POST-EXERCISE KETOSIS IN NON-DIABETIC SUBJECTS

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CONTENTS

FOREWORD

This thesis has been written so that the chapters follow logically one after the other; however, attempts have been made to make each chapter as self-contained as possible, with a description of why the work was undertaken, the methods used, the results obtained, plus a discussion of the significance of the results in the context of existing knowledge. The casual reader would therefore be able to read Chapter 8, The Synopsis and General Discussion, for instance, without having to refer to the detailed descriptions of the experiments in the preceding chapters.

Every attempt has been made to avoid unnecessary and irritating abbreviations. Complete eradication of this evil has however been impossible, especially in the diagrams and tables, but these are explained as far as possible in the text so that the reader is not repeatedly forced to refer to a glossary. The following is a list of the abbreviations used in the body of the thesis; the abbreviations used in the Appendices are explained before each set of tables.

'Ketone Bodies' (sometimes referred to as just 'ketones') are taken to mean acetoacetate and D-3-hydroxybutyrate. No account is taken of acetone, which is formed by non-enzymatic breakdown of acetoacetate, and is not thought to be of physiological importance.

SD: Standard Deviation.

SEM: Standard Error of the Mean.

P: Probability.

h: Hour(s).

min: Minute(s).

ATP: Adenosine Triphosphate.

-CoA: Coenzyme A.

FFA (also referred to as 'free fatty acids'): Long-chain free fatty acids.

HGH (also referred to as 'growth hormone'): Immunoreactive Human Growth Hormone.

IRG (also referred to as 'glucagon'): Immunoreactive Glucagon.

IRI (also referred to as 'insulin'): Immunoreactive Insulin.

3-HB:AA: 3-hydroxybutyrate:acetoacetate molar ratio in the blood.

et al.: and others.

Fig.: Figure.

Otherwise the standard SI units and symbols have been used.

Declaration:

This thesis is the original work of the author, both in concept and in its execution. The results of the work and ideas of others, mentioned in the text, are fully referenced.

Portions of the work described in this thesis have already been published:

Koeslag, J.H. Daily blood ketone body concentrations after acute exercise. S.Afr. Med. J. (1980) 57: 125-127.

Koeslag, J.H., T.D. Noakes, A.W. Sloan. Post-exercise ketosis. J. Physiol. (London) (1980) 301: 79-90.

ABSTRACT

The effect of exercise on the total ketone body (acetoacetate + D-3-hydroxybutyrate) concentrations in the blood was studied to find out whether the susceptibility of non-athletes, compared with athletes, to develop post-exercise ketosis (166) is the result of the former's increased reliance on glycolysis during exercise.

In the first experiments, use was made of the diving reflex (13) to induce peripheral vasoconstriction during exercise in both physically trained and untrained subjects. It was hoped that under these circumstances athletes and non-athletes would utilize similar amounts of muscle glycogen during exercise, and therefore develop similar degrees of ketosis after exercise, if the glycogen content of the muscles was in fact the factor which determined post-exercise ketosis.

Ten non-athletic subjects, six long-distance runners, and three competitive swimmers were therefore studied before, and for 9½ hours after swimming in the early morning. The last meal was eaten during the evening before the swim. On the first test day the subjects swam underwater for as far as they could go three times in succession. A week later the same distance was swum on the surface without breath-holding.

There was no increase in the post-exercise blood ketone body concentrations in any of the subjects after either form of the exercise, compared with control day values (when the subjects fasted, but did not swim at 07h30). Similar results were obtained when healthy young medical students (aged 18 - 23 years; trained and untrained) performed maximal exercise for 15 minutes, or moderate exercise for up to 90 minutes, on a bicycle ergometer.

When six older subjects (aged 30 - 51 years) exercised at 75 W for 90 minutes, three of them developed ketonaemia, which reached its maximum intensity about three hours after exercise. The exercising heart rates of these older subjects were similar to those of the younger non-athletic subjects who had performed the same

exercise, but had not developed post-exercise ketosis.

An extra 60 - 90 g sucrose in the diet of the subject who had developed the most marked post-exercise ketonaemia, abolished the response, whereas carbohydrate restriction intensified it. A protein-fat diet caused two well trained marathon runners to develop the highest post-exercise blood ketone body levels yet recorded (3,88 mmoles/l). Free fatty acid, glucose, growth hormone and insulin concentrations in the serum followed patterns different from the ketone body levels during, and for 7½ hours after exercise, but were also affected more by diet than by training. Post-exercise ketosis, previously ascribed to a lack of athletic training, could equally well be ascribed to the lower carbohydrate intake of sedentary subjects compared with athletes: the two marathon runners were estimated to eat about twice as much carbohydrate in their regular diet than the sedentary subject who had developed post-exercise ketonaemia without carbohydrate restriction.

The final experiments were designed to find out whether post-exercise ketosis was the result of the low levels of glycogen in the body, or of the gluconeogenesis which occurs after exercise to replenish the carbohydrate stores.

Twenty-four highly trained athletes were therefore studied after prolonged exercise following a protein-fat diet to induce post-exercise ketosis. Six of them were then given 100 g alanine to take by mouth, six ingested 100 g glucose, six ingested 100 g starch, and the remaining six acted as controls. It was found that both alanine and glucose ingestion reduced the blood ketone body concentration from about 2 mmoles/l to less than 0,4 mmoles/l in 3 hours. Starch had a minimal effect on the blood ketone body levels during the 5-hour observation period. Alanine and glucose exerted their antiketogenic effects in the context of widely different serum insulin, glucagon and growth hormone concentrations. Similar results were obtained in starvational ketosis, and even in normoketonaemic subjects.

The results indicate that ketogenesis is not the result of

gluconeogenesis, nor of a low insulin/glucagon (+ growth hormone) ratio in the blood. It is concluded that low levels of glycogen, or of a metabolic intermediary of glycogen metabolism (such as glucose-1-phosphate, or glucose-6-phosphate) in the liver is probably the single most important stimulus for ketogenesis after exercise and starvation.

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CHAPTER 1

INTRODUCTION

Most physiological, endocrinological, and biochemical parameters which change during exercise revert, after a short overshoot, fairly rapidly to their resting level when the increased muscular activity ceases. Since this is only to be expected, relatively little attention has been paid to the recovery phase of exercise.

That the post-exercise period might hold some surprises, however, is indicated by the behaviour of the blood ketone body concentrations after exercise, which, instead of returning to normal, tend to deviate progressively further and further from the resting level as time elapses after exercise. It is not yet certain how long this continues, but what is certain is that the metabolism undergoes profound changes after being challenged by exercise, and that these changes are worthy of investigation, not only for their own sake, but also for the light they may throw on the understanding of ketonaemia in general.

Post-exercise ketosis has been under sporadic investigation from about the beginning of the century, but attracted little attention until R.H. Johnson and co-workers in Glasgow (46,61,68,162, 166,167,168,169,170,171,172,173,174,175,176,177,178,259,268,271, 272,273) rescued it from obscurity with their intensive investigations during the 1970's. As a result, the subject has now been mentioned in recent editions of some of the standard physiology textbooks (33, 263).

Historical Review

In 1909 Forssner (106) reported finding increased quantities of acetone in his urine on days when he had undertaken a brisk walk in the early morning. The experiments had been undertaken to study protein metabolism during exercise on a low carbohydrate diet (55 - 60 g carbohydrate per day. Total energy

intake about 14 MJ per day). After 14 days on this diet, when the daily excretion of acetone was stabilized, he started his exercise experiments of walking 4 km in 36 minutes at 06h30. This was followed by bed rest till 09h30 and then work in the laboratory. On the days that he exercised the excretion of acetone in the 11h30 - 13h30 portion of urine was always found to be markedly raised. Although there was less acetone in the next urine portions, the acetonuria remained elevated above control day levels for 2 - 3 days. Forssner found that whereas 15 g glucose caused a rapid decrease in acetonuria on control days, it had no effect if taken immediately after exercise.

Fourteen years earlier, in 1895, Hirschfeld (144) had made a systematic study of the factors which influenced acetone excretion in non-diabetic subjects, but failed to demonstrate any noticeable change in acetonuria as a result of exercise. His subjects for the exercise experiments were two labourers who were admitted to hospital with shortness of breath and coughing in the one case, and 'light rheumatic pains' in the other. After 10 days on a high protein, low carbohydrate diet the two subjects were asked to scrub and clean some outhouses, and then to turn the handle of a centrifuge, from 08h00 to 12h00. They found this work extremely tiring; and their exercising pulse rates were over 120 beats per minute. However, neither the 08h00 - 12h00, the 12h00 - 20h00, nor the night urine contained more acetone than on control days, in either subject.

In 1911 Preti (265) noticed increased quantities of acetone in the urine of phlorhizin diabetic dogs for several days after exercise. These dogs were on a constant diet of beef. When a non-diabetic human subject on a high protein diet (100 g meat extract, 3 eggs, 100 g chicken per day) performed exhausting exercise by running up and down stairs, a large increase in acetonuria was noticed.

In 1934 Gemmill (116) reported that, whereas post-exercise ketonaemia could not be demonstrated in three healthy subjects on a normal diet, ketone body levels in the blood did rise significantly after exercise (50 W for 1 hour) if the subjects had been

on a low carbohydrate diet for 4 days. The maximum blood ketone body concentrations occurred about 2 hours after work.

In 1936 Courtice and Douglas (69) were studying the changes in respiratory exchange rate and respiratory quotient (RQ) during and after submaximal exercise, when they noticed that Rothera's test of the urine was frequently positive after exercise, though never before or during exercise. The exercise chosen for their experiments was a 16 km walk at 7,2 km/h, which was performed alternately by Courtice, aged 24 years, and by Douglas, aged 52 years. Courtice sometimes did not develop post-exercise ketonuria, but if he 'somewhat restricted his carbohydrate intake on the day preceding the test' it was always present. Conversely, if either of them increased their carbohydrate intake on the day before the walk, ketone bodies would not appear in the urine after the exercise. The appearance of the ketone bodies in the urine depended on whether or not the RQ fell to below 0,76 on cessation of the exercise.

Eating breakfast (eggs, bacon, toast, jam and tea), or taking 50 g glucose with 40 g bread, immediately before exercise, had no measurable effect on the post-exercise ketonuria. Fifty grams of glucose taken immediately after exercise did not abolish the development of post-exercise ketosis (70), which was in accordance with Forssner's findings (106) in 1909; but, if the sugar was taken 1½ to 4 hours later, there was a prompt reduction in the excretion of ketone bodies. The ketonuria could also be abolished by an injection of insulin (3 units intravenously) without taking sugar (70,241).

The glucose tolerance curve immediately after exercise was not significantly different from normal (70), but when the glucose administration was delayed for 1½ to 2 hours, a reduced tolerance was seen, similar to that obtained when the subject had been on a low carbohydrate diet (55 g carbohydrate per day; total energy intake 11 MJ per day) for 9 days, without performing strenuous exercise (69,70,241).

Most of the observations were discontinued 3 hours after

exercise, but on the one occasion that Courtice's urine was tested for more than 9 hours, the excretion of ketones was found to be biphasic, with an early peak at about 2 - 3 hours after the walk, followed by a dip and a second peak 5 - 6 hours later (69).

When the walk was interrupted by 30 minutes of rest every 6,4 km, the ketonuria appeared during the second rest period. It remained unchanged when the walk was resumed, but intensified again during the next rest period. A similar pattern was observed when a 10 km walk was undertaken 2 hours after a 16 km walk (69).

When the same exercise was performed by Mills (241) in 1938, with Douglas acting as control, no post-exercise ketonuria or reduced glucose tolerance could be demonstrated, even after carbohydrate restriction; this was ascribed to the 'large proportion of carbohydrates in Mills' regular diet'.

Drury (78) found that exercise produced no noticeable increase in the total daily urinary ketone body elimination, but bed rest increased it by almost 100% in a subject who had been on a strict 'ketogenic' diet for 3 weeks. Repetition of the experiment (79) showed, however, that there was a marked difference between the normal day- and night-time excretion of ketone bodies. Thus, while 2 hours of playing tennis 'at some time during the day' did not significantly affect ketone body excretion between 08h00 and 20h00, there was a marked increase in the ketone body elimination at night. Bed rest abolished the normal day-night rhythm by raising the day-time excretion of ketone bodies to that normally found at night.

Observations on groups of rats (80) which were made to swim for a total of 10 minutes(5 two-minute periods of exercise interspersed with 1-minute periods of rest), showed that on a normal diet blood ketone body levels started to rise soon after completion of the exercise, and were still rising 3½ hours later, when observations were discontinued. When the rats were already ketonaemic due to fasting, the swim lowered the blood

ketone body concentrations, but they rose again afterwards, to overshoot the control values by about 40% before returning to these values at about 6 hours after the swim.

Neufeld (247), in 1943, confirmed that blood ketone body concentrations decreased during exercise, but rose sharply after exercise. When the exercise was resumed 105 minutes later, the pattern was repeated, only at higher blood ketone body levels.

In 1947 Heilesen (138) found that the blood 3-hydroxybutyrate concentration rose during exercise (176 W for 1 hour), and reached a peak 15 minutes after exercise. Thereafter it fell to a plateau at about twice the normal concentration. Gammeltoft (114), in 1952, made a similar observation, but showed that this peak in the immediate post-exercise blood 3-hydroxybutyrate level was probably due to an acute change in the Redox state of the body, as there was a concomitant fall in acetoacetate concentration; the total blood ketone body concentration rose only slightly during this period.

In 1954 Grollman and Phillips (129) found that the immediate post-exercise blood ketone body concentrations of physically exhausted rats were much higher in physically trained (mean 8,98 mg/100 ml; range 5,43 - 14,80 mg/100 ml) than in untrained animals (mean 4,32 mg/100 ml; range 1,93 - 6,17 mg/100 ml). The resting blood ketone body concentrations were not affected by training (1,56 - 1,77 mg/100 ml).

Similar results were obtained by Passmore and Johnson (261) in 1958. Ten healthy human subjects, 3 of whom were in 'full training' for university rugby or squash, were given a balanced 12,6 MJ/day diet for 48 hours, after which they walked 16 km on an indoor treadmill at 6,7 km/h, and then lay down for 6 hours. In a warm environment (21^{o} - 25^{o}C) the athletes excreted an average of 2,3 mmoles total ketone bodies in the urine during the 6 hour recovery period, compared with 1,1 mmoles excreted by the non-athletes. In a cool environment (8^{o} - 14^{o}C) the values for the athletes and non-athletes were 3,1 and 2,5 mmoles

respectively. The blood ketone body concentrations 5 hours after exercise were 0,98 and 1,04 mmoles/l for the athletes in warm and cool conditions respectively; and 0,68 and 1,28 mmoles/l for the non-athletes. The authors (164,165,261,262) were able to account for most of the variations in post-exercise urinary ketone body elimination by means of a formula which incorporated water balance and the proportion of the total energy output derived from fats during the walk (coefficient of correlation = +0,95). The athletes' increased urinary excretion of ketone bodies appeared to be related to the greater proportion of fats which they had utilized during the walk.

In contrast, Johnson et al., in 1969, published the first (166) of a series (46,68,162,169,171,173,176,178,271,272) of articles which showed that post-exercise ketonaemia was more marked in non-athletes than in athletes. When 9 athletes and 18 non-athletes ran at their own speed on an outdoor track for 1½ hours, there were striking differences in the blood metabolite concentrations of the two groups (166). Blood ketone body concentrations started to rise during exercise, and in the untrained group reached a mean value of more than 1,6 mmoles/l at 1½ hours after exercise, compared with about 0,3 mmoles/l in the athletes. It was noted that whereas the blood glycerol levels rose to almost the same extent in the two groups, the plasma free fatty acid concentrations were significantly higher in the untrained group than in the trained group during exercise, and continued to rise for at least another hour during recovery. Blood lactate and pyruvate levels rose during the first 30 minutes of exercise in the untrained group, but not in the trained group; at the end of the run the concentrations of these two metabolites were normal in all subjects. It was concluded that, although both groups had apparently mobilized fats to the same extent during exercise (as evidenced by the equal rise in blood glycerol level in the two groups), the non-athletes had failed to utilize their free fatty acids as effectively as the athletes. The resulting high levels of free fatty acids in the blood had stimulated ketogenesis in the sedentary subjects.

Since these results might have been due to the non-athletes

working relatively harder than the athletes (the mean heart rates after 1 hour of running had been 165 beats/minute for the non-athletes, and 130 beats/minute for the athletes), further experiments were carried out (162,271). Four racing cyclists and five untrained subjects were studied during and after 20 minutes strenuous exercise on a bicycle ergometer, with the work load individually adjusted so that each subject worked at a heart rate of between 150 and 170 beats/minute. The trained cyclists worked at an average of 175 W for the last 10 minutes of exercise, and the non-athletes at 116 W, producing similar blood lactate levels (about 6 mmoles/l) immediately after exercise in both groups. Neither group showed a significant rise in blood ketone body concentration during exercise, but during recovery it rose in both. The rise was more marked, however, in the untrained group, in whom a mean concentration of about 0,18 mmoles/l was reached 150 minutes after exercise, than in the cyclists whose blood ketones did not rise above 0,07 mmoles/l ($P < 0,01$).

In another experiment 21 males, whose participation in sporting activities ranged from nil to university representation in running, ran 7 - 8 km on an outdoor track (171). Blood ketone body concentrations 2 hours after exercise were found to be negatively correlated with the subjects' fitness indices, as determined by the Harvard Step Test ($r = -0,52$; $P < 0,005$). There was no significant correlation between the post-exercise blood ketones and body fat content of the subjects. Total blood ketones 2 hours after exercise ranged in concentration from 0,05 to 0,43 mmoles/l, and fitness indices from 55 to 168.

This latter experiment (171) also showed that the apparent parallelism between the post-exercise plasma free fatty acid and ketone body levels which had been noted in the first experiment (166), was misleading, as the athletes in this experiment had higher free fatty acid levels (and lower ketone body concentrations) 1 hour after exercise than the non-athletes. The coefficient of correlation between the subjects' fitness indices and their 1 hour post-exercise plasma free fatty acid levels was +0,51 ($P < 0,025$).

When 8 young men underwent a 4-week physical training programme, the metabolic responses to exercise, including the phenomenon of post-exercise ketosis, changed from the typically non-athletic type to the athletic type, thus showing that the differences which had been observed between trained and untrainedindividuals was due to the habit of exercising, and not to an innate or genetic characteristic of athletes (272).

A similar experiment was performed by Winder et al. (356), who studied post-exercise ketosis in 6 healthy subjects before, during and after a 9-week endurance training programme. Resting blood 3-hydroxybutyrate concentrations did not change with athletic training, but the mean 60 minute post-exercise level fell from 0,42 mmoles/l to 0,20 mmoles/l in 3 weeks, after which there was little further change with continued training. Of the other parameters measured in this study, maximum plasma catecholamine and glucagon concentrations (during exercise) behaved similarly, while maximum glycerol and free fatty acid concentrations showed a more gradual decline during the whole 9-week training period.

Johnson et al. (46,168,170,172,174,177,271,272,274) attempted to find an endocrine cause for the ketonaemia after exercise in untrained subjects. Growth hormone, in particular, seemed to be a likely causative factor because of its known lipolytic and anti-insulin properties, and because of its tendency to rise to higher concentrations in the blood with exercise in untrained than in trained subjects (46,55,177,271,316,320). Six patients with hypopituitarism and 8 non-athletic control subjects were therefore studied before, during and after 30 minutes of exercise on a bicycle ergometer at a work load of 65 W (168,170). The serum growth hormone concentrations rose from about 5 ng/ml before exercise, to 35 ng/ml during exercise in controls, but remained at less than 1 ng/ml in the patients. Yet blood ketone body concentrations rose to higher levels in the patients than in the controls. When similar experiments were carried out on patients with acromegaly (174), blood ketone body concentrations decreased during the first 10 minutes of exercise, and then rose steeply to a peak (about 0,18 mmoles/l)

15 minutes later. The recovery phase saw a gradual fall in the blood ketone body concentration, quite unlike the normal response. It was clear therefore that growth hormone does not play an important role in the production of post-exercise ketosis.

Johnson et al. (169,173) found that post-exercise ketosis was due, in part at least, to a decreased utilization of ketone bodies during recovery. When 200 ml of a 0,4 M solution of acetoacetate (about 10 g acetoacetic acid) was taken by mouth before exercise, there was no statistical difference between the athletes' and non-athletes' rates of acetoacetate uptake and disposal. Exercise increased the acetoacetate tolerance of both groups to a similar extent. Acetoacetate tolerance was reduced during recovery, and this reduction was far more marked in the untrained group than in the trained group.

Glucose tolerance was found by Johnson et al. (167) to be reduced after exercise, confirming Courtice and Douglas' (69, 70,241) findings. Glucose tolerance was also reduced at the end of a 72 hour fast (70,167). In both, the blood ketone body concentrations fell when the glucose was administered, but the effect seemed to be more pronounced in starvational ketosis than in post-exercise ketosis. Forssner (106), and Courtice and Douglas (69), had also noted that post-exercise ketosis was more resistant than starvational ketosis to glucose administration, but in Johnson's experiment (167) the difference in response may have been due to the difference in blood ketone body concentration when the glucose was given (after starvation 2,82 mmoles/l; after exercise 0,60 mmoles/l).

The effect of diet on post-exercise ketosis was studied by Rennie and Johnson (175,273) in 6 long-distance runners, who ran 24 km in 90 minutes, first after eating their normal diet, and then after 'glycogen loading'. The 'glycogen loading' regimen consisted of 3 days of training on a high protein, high fat diet, followed by 3 days of rest on a high carbohydrate diet. Blood ketone body concentrations were similar at the start of both investigations, and also rose to the same extent

during exercise (to about 0,07 mmoles/l at the end of the 90 minute run). After the normal diet the blood ketone body concentrations continued to rise, reaching 0,25 mmoles/l at 105 minutes after exercise. After glycogen loading the ketone bodies remained constant after exercise. Blood lactate levels rose more during exercise after glycogen loading, than during exercise after a normal diet, as did the blood pyruvate concentration. The plasma immunoreactive insulin concentration was significantly higher at rest after 'glycogen loading' than after the normal diet. Exercise caused the plasma insulin concentration to fall in both situations, but the fall was less marked after glycogen loading. Blood glycerol, plasma free fatty acid and growth hormone concentrations during exercise were depressed by 'glycogen loading'.

Winder, Baldwin and Holloszy (355), found that post-exercise ketonaemia in rats was more pronounced in untrained than in trained animals, even when the latter ran at twice the speed of the former. Training was found to increase the acetoacetate oxidation rate twofold, and the 3-hydroxybutyrate oxidation rate threefold in gastrocnemius muscle homogenates obtained 24 - 72 hours after the last run (352,353,354,355). Levels of activity of the 3 enzymes involved specifically in ketone body oxidation were also increased: 3-hydroxybutyrate dehydrogenase activity had increased twofold, and acetoacetyl-CoA-thiolase had increased by approximately 55%. Other mitochondrial enzymes were increased to varying extents. No significant changes were found in the levels of these enzymes in heart muscle, liver, brain or kidney.

Askew, Dohm and Huston (14,81) investigated the interaction of diet, training and exhausting exercise on ketone body metabolism in rats. The training programme was similar to that used by Winder *et al*. (355) and produced the same enzymatic changes in skeletal muscle. There were three different diets: control (commercial laboratory chow), a high fat diet and a high carbohydrate diet. The nitrogen content per kilojoule of food energy was the same (1,3 g N_2/kJ) in all three diets. Exercise was continued to the point of complete exhaustion - the refusal

to run after electrical stimulation, failure to right themselves upon being placed on their backs and a deep body temperature of 40^{o} - $41^{o}C$. Resting plasma 3-hydroxybutyrate concentrations were significantly increased by training, and by a high fat diet. On all diets trained rats had a mean plasma 3-hydroxybutyrate concentration of about 2,1 mmoles/l when they were completely exhausted, compared with 0,8 mmoles/l in exhausted untrained rats. A high carbohydrate diet lowered the plasma 3-hydroxybutyrate concentrations at the end of exercise in trained and in untrained rats, and a high fat diet raised them, but neither diet changed the proportionality between the 3-hydroxybutyrate levels of the trained and untrained rats.

There have been many other reports of post-exercise ketosis in the literature, many of them as isolated findings which attracted sparse comment from the investigator(s). In other instances deliberate attempts to demonstrate post-exercise ketosis failed to show a significant rise in blood ketone body concentrations after exercise. These findings are summarised, together with the works reviewed in this text, in Appendix 1.

Discussion

No one has yet defined 'post-exercise ketosis'. The term has been used to describe an increased blood or urinary ketone body concentration immediately on cessation of exercise, or to an increased blood or urinary ketone body concentration during the recovery period - the usual standard for comparison being the pre-exercise ketone body concentration. Semantically, the term could apply to both situations, but from a functional point of view, any observation made at the end of exercise is still part of the exercise period, since, if the exercise had been continued for another 1, 5 or 10 minutes, the observation, and its significance, does not change, but it would no longer be considered to be 'post-exercise'.

In this thesis, therefore, the term 'post-exercise' will be

used to refer to events occurring during recovery, after exercise has stopped; and the words 'immediate post-exercise' will refer to the moment that exercise ceases. 'Post-exercise ketosis' is then defined as an increase in the blood or urinary ketone body concentration during the recovery phase - with the immediate post-exercise ketone body concentration serving as datum point - if appropriate control studies are not available for the comparison.

Blood ketone body concentrations have been found to decrease during recovery in only two instances: in a group of patients with acromegaly studied by Johnson and Rennie (174), and in a group of trained rats studied by Winder, Baldwin and Holloszy (355). In the former, the blood was taken for analysis every 30 minutes for 90 minutes, and in the latter only once at 60 minutes. The possibility of an early, missed, post-exercise peak in blood ketone body levels is therefore not ruled out, especially in the latter investigation. In all the other investigations blood and urinary ketones either increased after exercise, or, occasionally, remained unchanged. The individual variability, and even the variability shown by the same subject repeating the same exercise (69), is very marked. Thus Johnson et al. (68,169) reported total ketone body concentrations which ranged from 0,4 to over 2,5 mmoles/l at 90 minutes after a 1½ hour run in individual untrained subjects. Most investigators have found that blood ketone body concentrations rarely rose higher than 1,0 mmoles/l at 1 hour after exercise, and usually remained below 0,4 mmoles/l. These values are within the range of normal (1,163,338,341,345), making it doubtful whether post-exercise ketosis is possibly as common as the many references to the phenomenon would indicate, especially since few of these investigations have been accompanied by suitable control (no exercise) studies.

The factors which enhance or suppress the ketogenicity of the post-exercise period are still far from clear. The composition of the pre-exercise diet would appear to be very important: a low carbohydrate diet enhancing post-exercise ketosis (14,69, 116,247), and a high carbohydrate diet reducing it (14,69,273).

Yet Hirschfeld (144) missed being the discoverer of post-exercise ketosis in 1895 in spite of the almost carbohydrate-free diet which his subjects had taken for 10 days before the exercise. Drury (78,79) and Barnes et al. (30) also found that a 'ketogenic diet' gave equivocal post-exercise ketone body results. Passmore and Johnson (164,165,261,262) found that high environmental temperatures, giving rise to increased sweat rates during exercise, reduced the level of post-exercise ketosis. But Åkerblom (1) found that the seasons in Finland had no influence on the blood ketone body levels of exercising children; and Kuroshima et al. (201) found that the ketosis of rats which had swum for 30 minutes was not affected by the temperature of the water. Obesity has an antiketogenic effect on nutritional ketosis (125,126,128,184,262), but does not influence post-exercise ketosis (171).

It is difficult to assess, from the wide variety of exercises used in the study of post-exercise ketosis, which form of exertion is the most ketogenic. Even when the same exercise was employed by the same investigator, highly variable results were obtained (68,69,85,166,167,169,173). A general impression would indicate however that prolonged moderate exercise (e.g. walking) tends to give rise to post-exercise ketosis more readily than intense exertion of short duration.

Of the pathological conditions which are most likely to predispose to post-exercise ketosis, uncorrected insulin-dependent diabetes mellitus (37,194) is probably the most prominent. Here exercise may precipitate hyperglycaemic ketoacidosis (37, 95,142,332). Hypopituitarism was found by Johnson et al. (168, 170) to give rise to higher post-exercise blood ketone body concentrations than in normal controls. Acromegaly was associated with a suppression of post-exercise ketosis (174). Alcoholism enhanced post-exercise ketosis (61).

In contrast with the recovery phase, the exercise period itself tends to be antiketogenic (24,30,46,60,79,80,114,134,150,164, 247,301,353). In many cases it results in a fall of blood ketone body concentrations, particularly if the levels were

already high at the beginning of exercise due to a low carbohydrate diet (80,114,150), or to previous exercise (247). When the blood ketone bodies do increase during exercise the rise is smaller than would have occurred if an equivalent energy deficit had resulted from starvation (164). Even the ketosis of diabetes mellitus, if there is some insulin present, appears to be ameliorated by exercise in some cases (1,142,194, 332) but not in others (37,134,142,189). This effect of exercise appears to be due, in part, to the lactacidaemia of exertion (149,150) which has an antilipolytic effect on adipose tissue (9,51,108,149,161,240) and possibly an antiketogenic influence on the liver (149). The rate of ketone body disposal from the blood is also increased during exercise (24,82, 99,169,173,312), but it is not always the exercising skeletal muscles which take up the ketones; in fact, Hagenfeldt and Wahren (131,132) have demonstrated a net release of acetoacetate or 3-hydroxybutyrate, or both, from exercising forearm muscles. Similar results have been found by Dieterle et al. (76) and by Rennie et al. (274). Hind limb perfusion experiments, however, have shown that skeletal muscles can utilize ketone bodies as fuel, especially if the arterial concentration is high (44,123). In normal subjects the contribution of ketone bodies to the total fuel budget is negligable (24,131,132,280), but in diabetes (30,301,332), and possibly in starvation (252), they may constitute a significant source of energy, accounting for 6% or more of the CO_2 production during exercise (99).

Many of the more recent investigations into post-exercise ketosis have concentrated on the antiketogenic effects of endurance training, first described by Johnson et al. (46,68, 162,169,171,173,178,271,272), and confirmed by Winder et al. (355,356); though an earlier report by Passmore et al. (261) had shown that athletes were more prone to post-exercise ketosis than normal controls. Much of this research has concentrated on the fuel-hormone response to exercise (130,166, 170,171,172,174,271,272,356), and on the training-induced alterations in muscle enzyme activities (352,353,354,355), to find the cause of this phenomenon. Summarising the results

of these studies in 1978, Holloszy, Winder, Fitts and Rennie (147) concluded that 'among the factors that appear to contribute to the development of ketosis are an elevation of the plasma free fatty acid concentration, a decrease in plasma insulin concentration, a rise in plasma glucagon concentration, and a depletion of liver glycogen..... Trained individuals exhibit less of a decrease in plasma insulin and a smaller elevation of plasma glucagon during and after exercise than do untrained subjects. Trained individuals are also protected against liver glycogen depletion during exercise, and tend to have lower plasma free fatty acid concentrations during and after exercise than do untrained subjects. In view of these differences, it seems likely that a lower rate of hepatic ketone synthesis during and after exercise plays an important role in accounting for the smaller increase in plasma ketone concentration seen in the trained as compared with the untrained state'. This conclusion, and the findings on which it is based, is discussed more fully in Chapters 6, 7 and 8.

The duration of post-exercise ketosis is not known. Most of the more recent studies have not been continued for longer than 90 minutes after exercise, at which time the blood ketone body concentrations were still rising. Eriksson et al., (85), however, found that blood ketone bodies reached their peak concentration at 1 hour, and were clearly falling at 1½ hours, after exercise. In an anecdotal reference to post-exercise ketosis, Ballasse (25) said that "the peak (post-exercise ketone body levels) occurred 2 - 3 hours after exercise". The rate of urinary excretion of ketone bodies in Passmore and Johnson's study (261) was still increasing 4 - 6 hours after work. Courtice and Douglas (69) found two peaks in Courtice's post-exercise urinary ketone body excretions: one at 2 - 3 hours, and a second at 7 - 9 hours after exercise. Forssner (106) also found that the excretion of acetone in the urine was biphasic on exercise days; the first peak occurring at about 5 - 6 hours after exercise, and the second at 12 hours. On a low carbohydrate diet, the post-exercise ketonuria remained higher than on control days (also on a low carbohydrate diet, but no exercise) for about 72 hours (106,265).

Conclusion

Post-exercise ketosis appears to be a very variable phenomenon. Some of the variation is undoubtedly related to the differences in the composition and quantity of food consumed by the subjects in their usual diets over the past 85 years, but other factors over which there has also been little control, are the environmental temperature and the daily basal physical activity of the individuals studied. Whether age, time of day at which the exercise was performed and habits such as smoking, and alcohol and caffeine consumption, have influenced the results is not known.

CHAPTER 2

BLOOD KETONE BODY AND LACTATE CONCENTRATIONS ON NON-EXERCISING DAYS

Circadian variations in blood acetoacetate and 3-hydroxybutyrate concentrations are quite marked in non-fasting subjects, with peaks occurring approximately 2 - 4 hours after meals (i.e. at about midday, 17h00 and at 23h00) (326,327). Similar daily variations have been found in the acetone content of the expired air (313), and in the rate of ketone body excretion in the urine (32).

The subjects who took part in our exercise experiments, ate no food from the evening on the one day, till 17h00 on the next day: an approximately 24-hour fast. Normal values had to be established for these conditions, as well as for the possible influence of the weather in Cape Town, where the studies were conducted.

Method

A. Normal Daily Activities

Thirty-four healthy male subjects, most of whom also took part in the exercise experiments to be described later, were studied during a normal working day from 07h30 to 17h00, having had their last meal during the previous evening. No food was eaten during the observation period, but energy-free beverages were taken ad libitum. No one smoked on experimental days.

None of the subjects had a history of chronic illness, a change in body mass or of hormone therapy during the previous year. Eighteen of the subjects took part in regular physical exercise (running at least 5 km on not less than 4 days in the week), though not on the observation day. The remainder did not take part in any sporting activity. Their ages, heights and masses were as

follows:

		Age (years)	Height (cm)	Mass (kg)
Non-athletes n = 16	mean	20	176	69
	range	18 - 37	167 - 184	55 - 91
Athletes n = 18	mean	21	181	75
	range	18 - 29	172 - 192	61 - 101

Blood was taken by venepuncture at 07h30, 08h00, 09h30, 10h30, 13h00 and at 17h00 for the determination of the acetoacetate, D-3-hydroxybutyrate and L-lactate concentrations. The chemical analyses were carried out as described in Appendix 2.

These studies took place between the months of December and September.

B. Complete Physical Inactivity

Ten healthy male subjects, six of whom took part in regular sporting activities, were studied under the same conditions as in A, except that they were confined to armchairs for the whole of the observation period, being allowed up only to go to the toilet. Energy-free beverages were brought to them every 2 - 3 hours; there was no smoking. Their ages, heights and masses were as follows:

		Age (years)	Height (cm)	Mass (kg)
Non-athletes n = 4	mean	22	175	67
	range	20 - 27	165 - 183	64 - 72
Athletes n = 6	mean	20	182	82
	range	19 - 22	178 - 192	69 - 101

These studies were carried out indoors during June and July. Room temperature varied between 18^{o} - 20^{o}C.

Results

A. Appendix 3, Tables 1-2 to 1-5

The results of the experiments carried out during the warm months (December to March), and the cool months (April to September) are indicated for non-athletes and athletes in Figures 1 - 3.

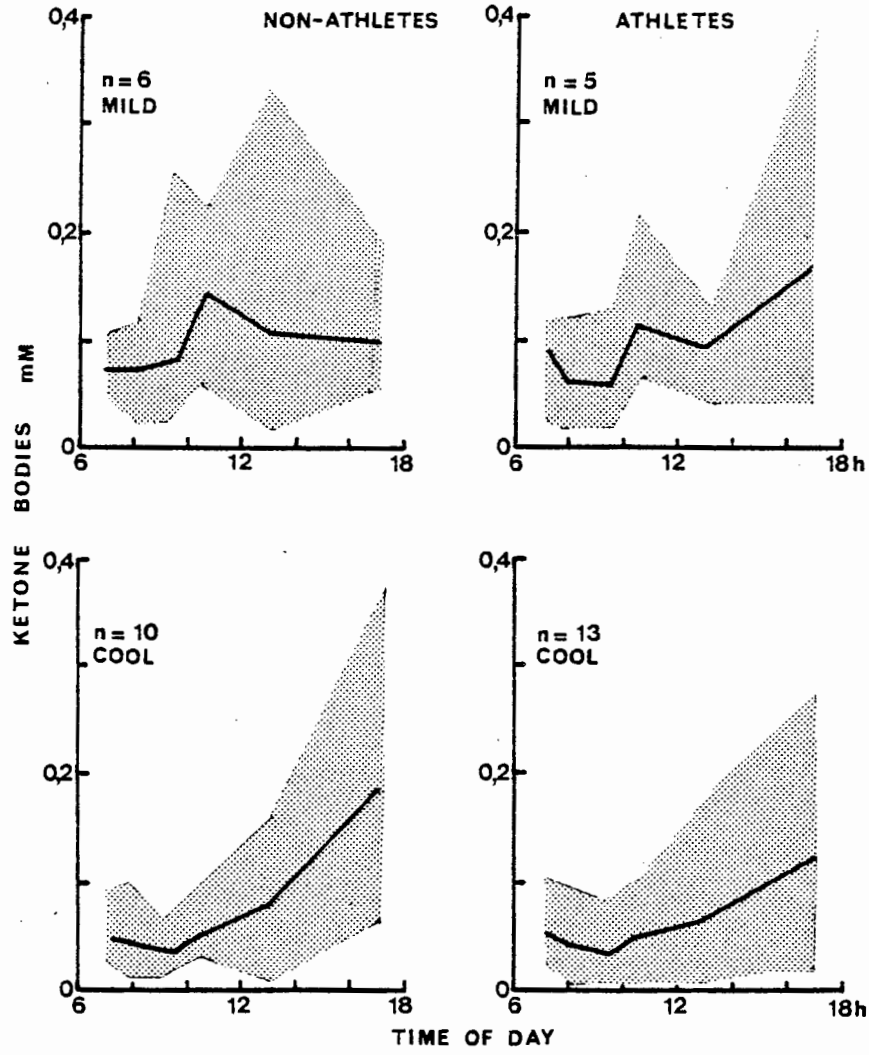

Fig. 1. The mean (continuous line) and range (stippled area) of the daily blood ketone body concentrations of 16 non-athletes and 18 athletes on ordinary working days during the mild months of the year (December-March) and during the cool months (April-September). No strenuous exercise was performed. The last meal was taken during the previous evening. No food was eaten during the observation period, but energy-free beverages were taken ad libitum.

The mean 08h00 outside air temperature on experimental days during the warm months was 18,5°C (range 14,5° - 21,2°C), and during the cool months 10,8°C (range 5,0° - 14,8°C). The mean 14h00 outside air temperature was 23,3°C (range 17,4° - 30,5°C) for the warm months, and 19,0°C (range 15,9° - 22,6°C) for the cool months*. However, the subjects spent most of their time indoors, where, although there was no air-conditioning, the temperatures were less extreme than outside. It did not rain on any of the experimental days.

* Data kindly supplied by the Weather Office, D.F. Malan Airport.

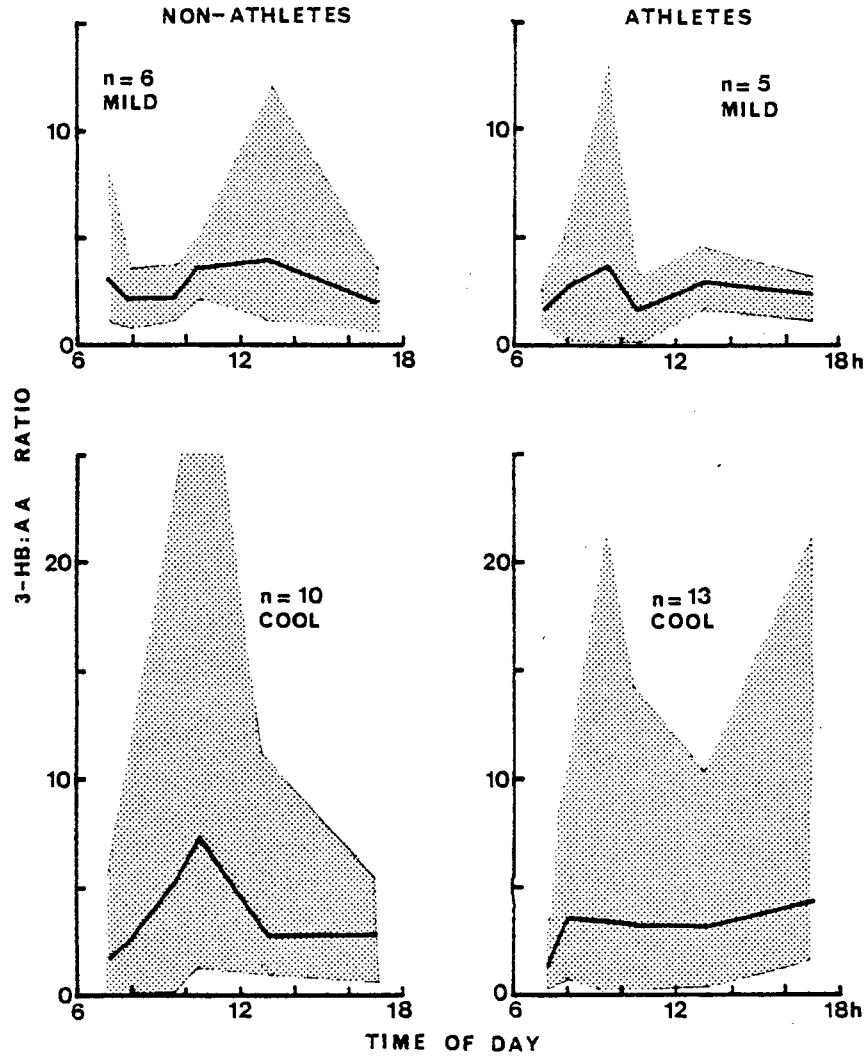

Fig. 2. The mean (continuous line) and range (stippled area) of the daily fasting 3-hydroxybutyrate:acetoacetate (3-HB:AA) ratios in the blood of non-athletes and athletes during the mild months (December-March) and the cool months (April-September) of the year. Experimental conditions are the same as in Fig. 1.

There was no significant difference between the blood total ketone body (D-3-hydroxybutyrate + acetoacetate) concentrations of athletes and non-athletes on non-exercising days, nor were there significant seasonal differences. Blood ketone body concentrations did vary significantly during the course of the day, tending to be lowest at 09h30 (mean 0,054 $\pm$ SD 0,063 mmoles/l), and highest at 17h00 (mean 0,142 $\pm$ SD 0,102 mmoles/l). This difference is highly significant ($P < 0,0005$; paired t test). Mean blood ketone body concentrations did not exceed 0,2 mmoles/l at any time of the day in any of the subgroups indicated in Figure 1, but individual values reached 0,4 mmoles/l at 17h00.

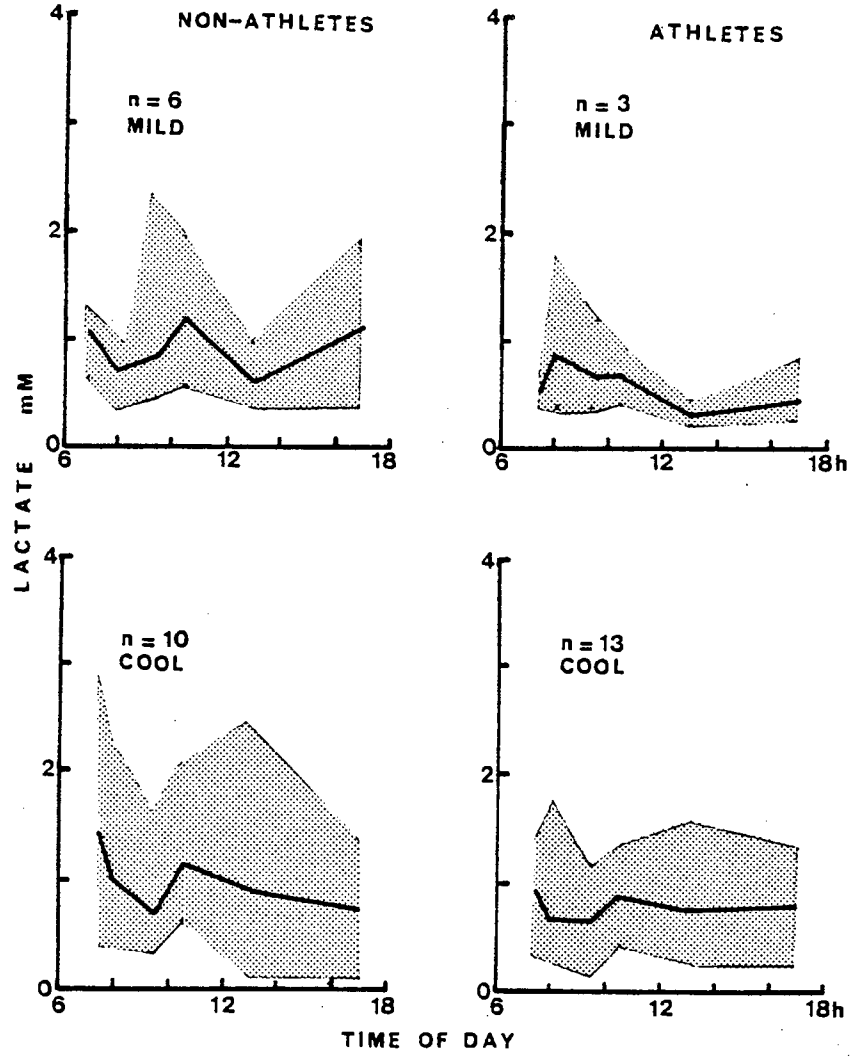

Fig. 3. The mean (continuous line) and range (stippled area) of the daily fasting blood lactate concentrations in non-athletes and athletes on ordinary working days without strenuous exercise. Experimental conditions and symbols are the same as in Fig. 1.

The 3-hydroxybutyrate/acetoacetate molar ratio showed considerable variability, but none that was significantly correlated with the time of day, athletic training or with the seasons of the year (Fig. 2). The mean 3-hydroxybutyrate/acetoacetate ratio, for all the control values, was 2,92 ($\pm$ SD 4,22).

Blood lactate concentrations showed no recognisable diurnal or seasonal pattern, but did differ significantly between athletes (mean 0,74 $\pm$ SD 0,37 mmoles/l) and non-athletes (mean 0,92 $\pm$ SD 0,57 mmoles/l), $P < 0,02$ - 2 tail t test (Fig. 3).

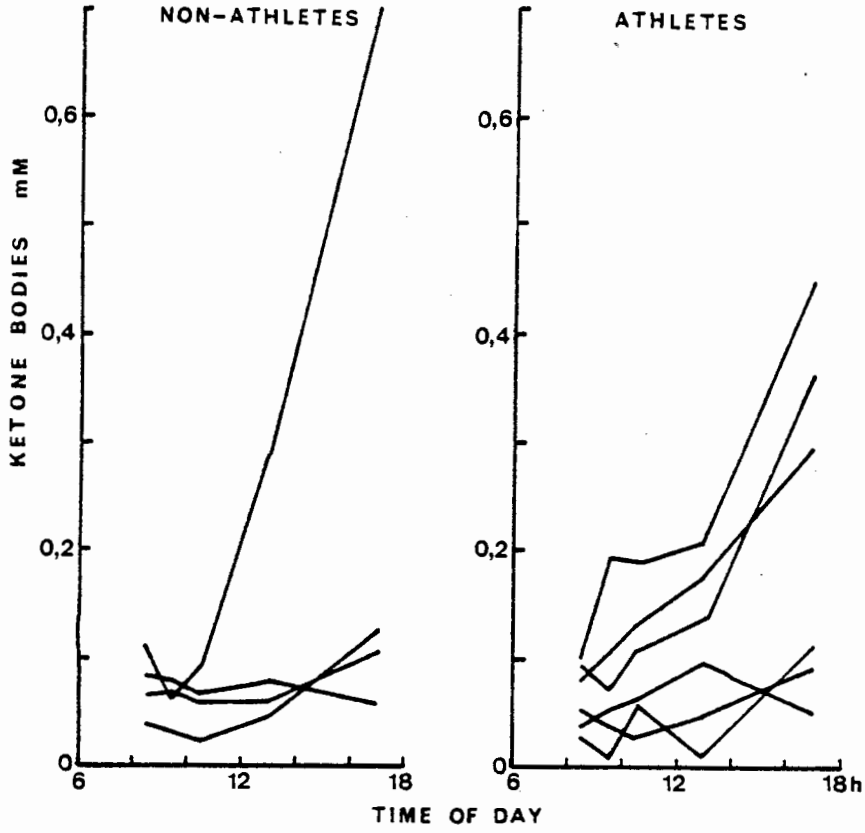

Fig. 4. The fasting daily blood ketone body concentrations of 4 non-athletes and 6 athletes who were confined to armchairs from 08h00-17h00. The last meal was eaten during the previous evening, and no food was taken during the observation period. Energy-free beverages were taken at 2-3 hourly intervals. The experiments were conducted indoors at air temperatures of 18°-20°C.

B. Appendix 3, Table 1-6.

When non-athletes and athletes sat completely still for 9½ hours, blood total ketone body concentrations rose markedly during the course of the observation period in 4 of the 10 subjects (Fig. 4). In one subject the blood ketone body concentration was found to be 0,702 mmoles/l at 17h00. There appeared to be no difference between the non-athletes' and athletes' blood ketone body response to complete physical inactivity.

The 3-hydroxybutyrate/acetoacetate molar ratio was not affected by physical inactivity (Fig. 5).

The blood lactate concentrations were again higher in the non-athletes (mean 0,81 ± SD 0,53 mmoles/l) than in the athletes (mean 0,67 ± SD 0,48 mmoles/l), but this difference

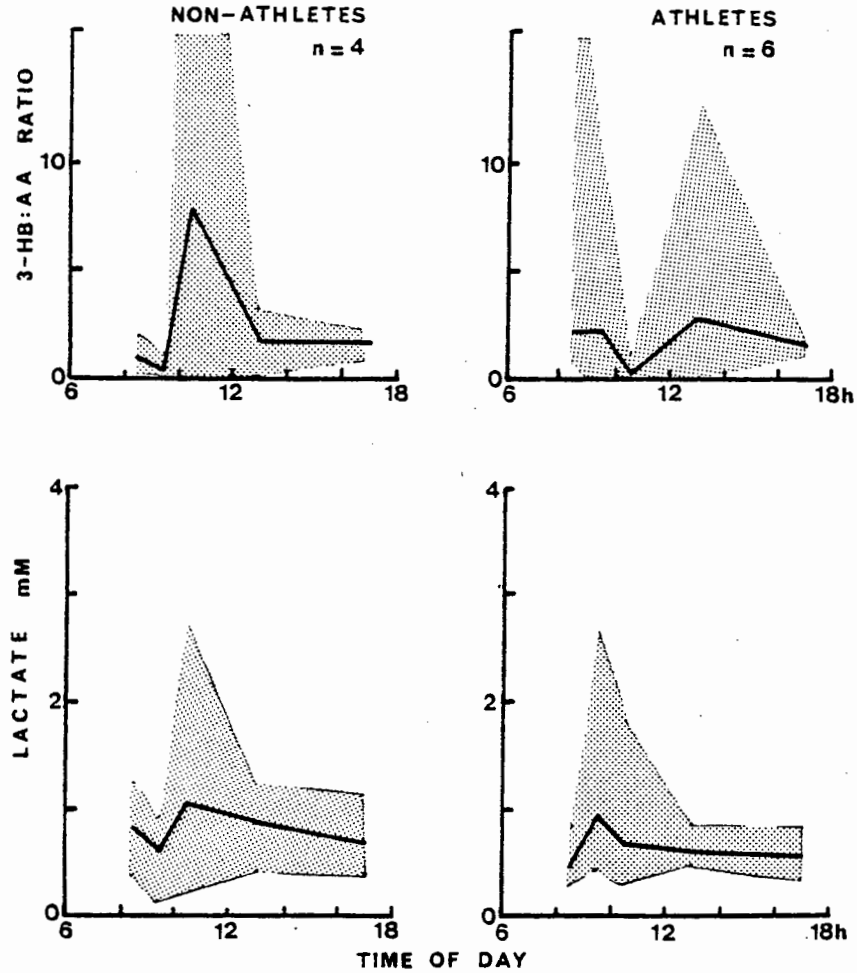

Fig. 5. The mean (continuous line) and range (stippled area) of the fasting daily blood 3-hydroxybutyrate:acetoacetate (3-HB:AA) ratios, and lactate concentrations of 4 non-athletes and 6 athletes who were confined to armchairs from 08h00-17h00. Experimental conditions are the same as in Fig. 4.

is not significant for the size of the samples (Fig. 5).

Discussion

Forssner, in 1909 (106), found that the urinary excretion of acetone on non-exercising days was cyclical, with a small peak at about midday, and a larger, broader peak during the evening and night (17h30 - 04h00). The lowest excretion rate occurred in the 06h30 to 09h30 portion of urine. Hubbard and Wright (151) also found the rate of urinary acetone excretion to be raised at about midday, but not at night. McClellan and Toscani (229) found, as Forssner (106) had done, that the daily acetone excretion had two peaks, but their subjects had all walked 5 - 8 km in the afternoon, so that the evening peak may in fact have been the result of post-exercise ketosis.

In all of these investigations the subjects had been on a low carbohydrate diet. In 1931, Behre (32) studied ten subjects on a normal diet, and found that the highest rate of ketone body excretion occurred during the afternoon and evening. The lowest rate of excretion occurred at night. Occasionally there were midday peaks. The omission of some, or of all, of the meals in the day did not alter the pattern.

Steward and Boettner (313) found peaks in the expired-air acetone concentration before breakfast, at about lunchtime and again in the early evening. This rhythm was abolished in diabetes mellitus. The circadian rhythm for the blood ketone body concentrations was discovered by Wildenhoff (341,342), who found that seven out of sixteen subjects had a small peak at noon. Fourteen of the sixteen subjects had raised blood ketone body concentrations at 17h00, and again at midnight.

The blood ketone body concentrations in our subjects, who did not eat during the day on which the observations were made, rose gradually from 09h30, when the lowest mean concentrations were recorded, till 17h00. Since no readings were taken between 13h00 and 17h00, it is possible that the dip in blood ketone body concentration which Wildenhoff (341) had found during this period, was missed. As in the other studies (32,151,341), our subjects did not all individually conform to the same general pattern. This might be due to the absence of cyclical variations in the blood ketone body concentrations of some persons, or it might be because the peaks are fairly narrow, and therefore likely to be missed if the blood samples are widely spaced through the day.

The cause of the daily rhythm in blood ketone body concentrations is not known. It is not directly the result of the pattern of meal times during the day, because the cycle was retained in the absence of meals (32), as in our experiments. It is possible, however, that it is the expectation of meals (frustrated, in this case) which raises the blood ketone body concentration; though, what purpose this would serve is not

clear. Complete physical inactivity, combined with fasting, also did not abolish the rhythm (Fig. 4). The possibility that the rhythm is the result of cyclical changes in the plasma levels of one of the blood hormones (e.g. insulin or growth hormone) was considered by Wildenhoff et al. (342), but they dismissed this as unlikely, though not on very firm grounds. The circadian variations in the blood ketone body concentration, whatever the cause, may be very marked however, and have to be taken into account in any work on ketone body metabolism.

The blood ketone body concentrations obtained in this study are similar to those found by Wildenhoff (341) and by Werk and Knowles (338) in non-fasting individuals, but are considerably lower than those found by Johnson, Sargent and Passmore (163) in 1958. Based on 198 observations in 99 airmen in the U.S.A. and 10 medical students in Scotland, these workers (163) found that the normal blood ketone body concentration was on average 0,71 (± SD 0,21) mmoles/l. The time of day is not stated in the report. Our mean values were never greater than 0,2 mmoles/l, though individual values frequently reached 0,4 mmoles/l; and only in one case did the blood ketone body concentration reach 0,7 mmoles/l, in a non-athlete after a day of complete physical inactivity (Fig. 4). Johnson et al. (163) found that summer and winter (in Indiana and Wisconsin) did not affect the normal blood ketone body levels, as was the case in our studies, but they did find that environmental temperature affected nutritional ketosis: ketonuria and ketonaemia being significantly greater in winter than in summer (288).

The range of normal blood ketone body concentrations reported by Williamson, Mellanby and Krebs (345) when describing the enzymatic determination of D(-)-3-hydroxybutyrate and acetoacetate used in our experiments, was from 0,046 to 0,876 mmoles/l. The time of day at which the observations were made is not mentioned. The mean 3-hydroxybutyrate/acetoacetate ratio was 2,73 (± SD 0,73), which is similar to our findings, as well as those of Gammeltoft (114), Wakil et al. (334) and Wildenhoff (341). Under normal circumstances

therefore, regardless of the time of day, about 70 to 75% of the blood ketone bodies consist of 3-hydroxybutyrate and the rest of acetoacetate, but wide individual variations can occur.

The blood lactate concentrations were significantly higher in the non-athletes than in the athletes, throughout the day (Fig. 3), even when the subjects were physically totally inactive (Fig. 5). This was true in most of our subsequent experiments as well. Yet, in Johnson's studies the athletes usually had higher mean pre-exercise blood lactate concentrations than the non-athletes (166,271,272), as was the case with Łukawaska and Połec's (213) trained and untrained teenagers. Karlsson et al. (182), Bjernulf et al. (41) and Le Blanc et al. (205) found that there was no significant difference between the resting blood lactates of trained and untrained subjects. On the other hand, Klausen et al. (191) found that the resting blood lactate concentration was significantly lower ($P < 0,05$) after five weeks of leg-exercise training than before, but was not affected by arm-exercise training. Penny and Wells (264) found that professional football players had significantly lower serum lactate levels (expressed in mg/kg body mass) than normal controls ($P < 0,01$). Houghton et al. (150) found that the resting blood lactate concentration was lower in trained than in untrained rats. It is clear therefore that athletic training does not consistently increase, or decrease, the resting blood lactate concentration, and that the differences which have been found between athletes and non-athletes in different studies were probably the result of some incidental factor.

Huckabee (152,153) found that the resting blood lactate concentrations were increased after meals, glucose infusions, anxiety, tensing of the muscles and hyperventilation. While any of these factors (except meals and glucose infusions) could have affected our results, it seems unlikely that they should always have affected the non-athletic subjects more than the athletes, when both groups of subjects were usually studied together, and were therefore presumably subjected to

the same stresses which resulted from having to be in the laboratory on time, and from the taking of the blood.

Kelman et al. (188) and Rennie et al. (175,273) have shown that resting blood lactate levels are increased in normal and in athletic subjects, by a high carbohydrate diet. Belo et al. (35) found that a high protein, low carbohydrate diet decreased blood lactate levels at rest. Since regular exercise increases the appetite (227), it is not unlikely that the proportion of carbohydrate in the diet might wittingly, or unwittingly, change, and thus give rise to an altered resting blood lactate level: the athletes in our studies presumably eating proportionately less, and in Johnson's studies proportionately more, carbohydrate in their diet than their respective normal controls. The tendency to eat more (or less) carbohydrate could depend on the particular dietary fad which is in vogue amongst athletes in a particular country at a particular time (74).

CHAPTER 3

THE EFFECT OF SWIMMING WITH AND WITHOUT BREATH-HOLDING ON POST-EXERCISE KETONE BODY CONCENTRATIONS

It is believed that a diminished reliance on glycolysis to supply the energy needed for submaximal exercise is one of the most important adaptations to occur in skeletal muscle after endurance training (145,146,147,183,196): muscle glycogen content decreases less rapidly in trained than in untrained muscle for the same work output (26,101,141,150,285). The need for gluconeogenesis during recovery (95,197,331), to replenish the muscle glycogen stores, is therefore smaller in the athlete than in the non-athlete. If gluconeogenesis is the stimulus for ketogenesis, as suggested by Krebs (198), and others (43, 86,115,211,212,315), this would explain Johnson's findings (68, 166) that post-exercise ketosis is more marked in non-athletes than in athletes.

The aim of this study was to test this hypothesis, using the diving reflex (13,54,298,362). Swimming under water elicits this reflex, which causes a drastic reduction of the blood flow to the limbs and exercising muscles, irrespective of the physical fitness of the subject (314). Muscle glycogen utilization would then be similar in trained and untrained subjects, and post-exercise ketosis, if it is determined by the glycogen content of the muscles after exercise, would be expected not to be different in the two groups. Swimming on the surface without breath-holding does not elicit the diving reflex, and the normal differences in muscle metabolism between trained and untrained subjects would be expected to become manifest again (16,59,148,297).

Methods

Nineteen healthy young male physiology students were used as subjects. Ten of the subjects did not take part in any form of regular sporting activity, six ran 5 km at least four times

a week but were not regular swimmers, and three were competitive long-distance swimmers in the Western Province Swimming Team. The three groups were designated: 'non-athletes', 'runners' and 'swimmers', respectively. A fourth group of six sedentary subjects, acted as controls for the non-athletes. All the subjects participated in the investigation voluntarily, after being fully informed of the intention, procedure and potential risks inherent in the experiments. The ages, heights and masses of the subjects were as follows:

			Age (years)	Height (cm)	Mass (kg)
Non-athletes	n = 10	mean	20	179	72
		range	18 - 29	176 - 185	64 - 81
Non-athletic controls	n = 6	mean	19	175	72
		range	18 - 20	167 - 181	63 - 77
Runners	n = 6	mean	20	178	72
		range	19 - 26	176 - 186	61 - 83
Swimmers	n = 3	mean	19	184	93
		range	19	179 - 192	79 - 101

The investigation consisted of two test days and one control day on which the athletes fasted from the previous evening till 17h00, but drank energy-free fluids ad libitum. Smoking was prohibited. The non-athletes were studied on the two test days, with a separate group of six subjects providing the control values. In all other respects the trained and untrained subjects were studied under identical conditions. On each observation day blood was taken be venepuncture at 07h30 and within ten minutes after exercise, followed by blood samples at 09h30, 10h30, 13h00 and 17h00 to coincide with the intervals between lectures and practical classes. The concentrations of D-3-hydroxybutyrate, acetoacetate and L-lactate in the blood were determined by the methods described in Appendix 2.

The non-athletes were paired off with the athletes (runners and swimmers), and on the first test day each pair swam underwater at 07h30 as far as the poorer swimmer of the pair was able to go, three times in succession, with two-minute periods of rest

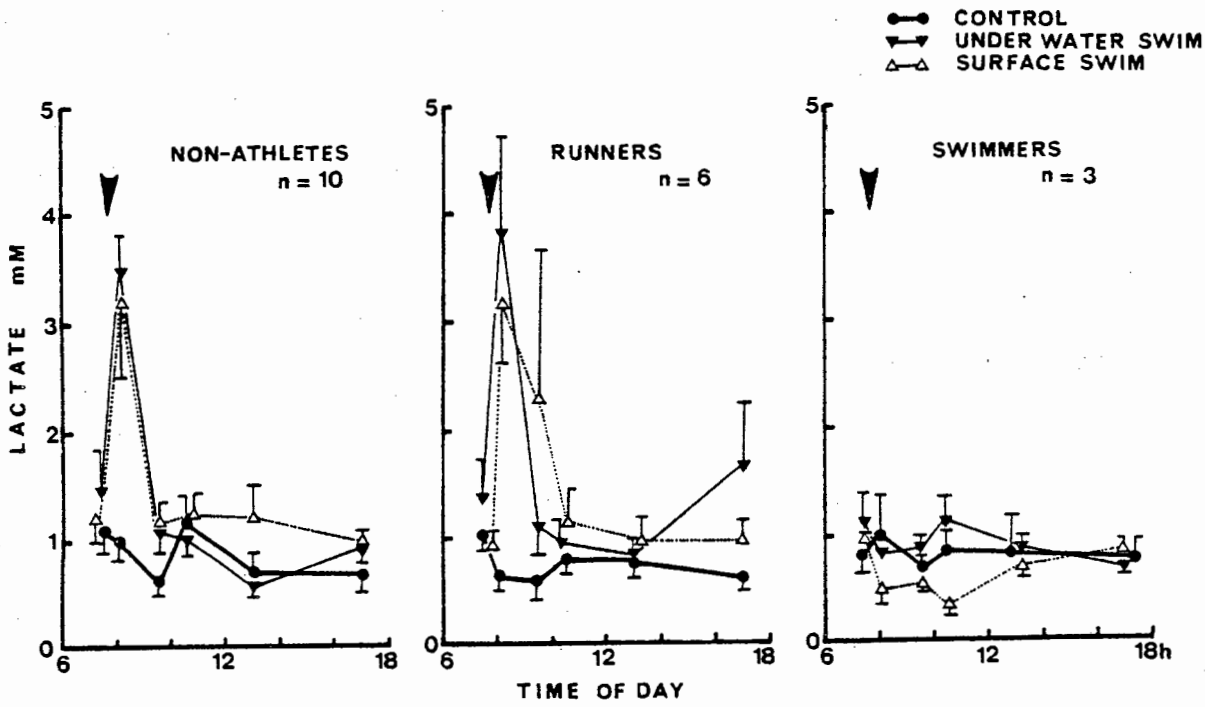

Fig. 6. The daily blood lactate concentrations (mean ± SEM) of 10 non-athletes, 6 long distance runners and 3 members of the Western Province Swimming Team who swam about 90 m with and without breath-holding at 07h30 (arrows). The last meal was eaten during the previous evening. No food was taken during the observation period, but all subjects performed their normal daily activities. No strenuous exercise was performed on the control day.

between swims. In practice this meant that the runners, whose underwater breath-holding stamina was less than that of the non-athletes, paced their sedentary partners, but vice versa for the swimmers and their partners, where it was the non-athletic partner who set the pace and distance of the swim. One runner felt faint after the swim and did not continue with the investigation, leaving an unpaired non-athlete to join another runner-non-athlete pair on the next test day.

On the second test day, a week later, the subjects swam the same distance as before, doing the same stroke, but this time on the surface of the water without breath-holding. The results were compared with the blood ketone body and lactate concentrations on a control day when the subjects did not swim in the early morning.

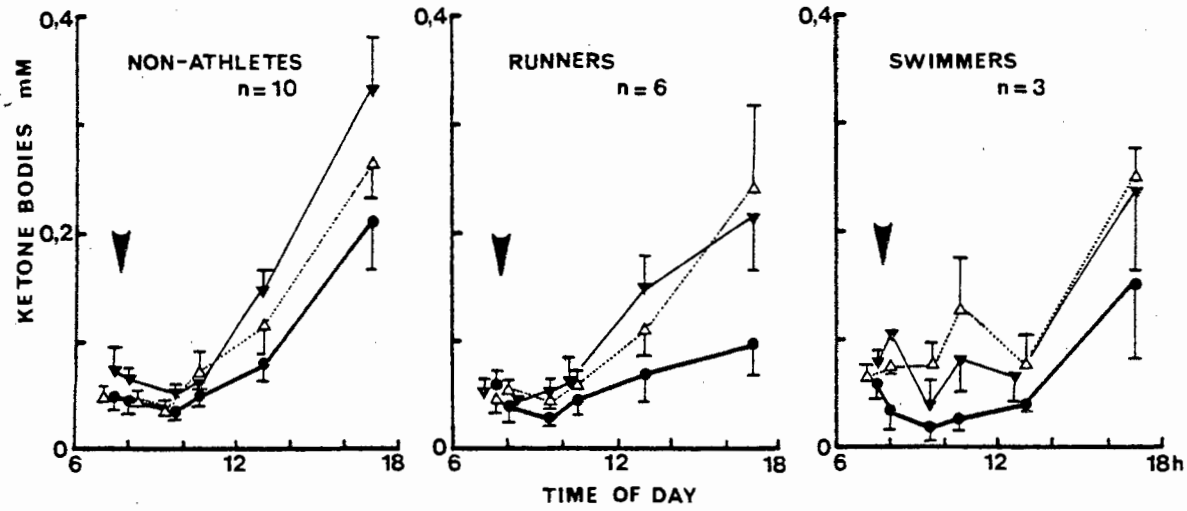

Fig. 7. The daily blood ketone body concentrations (mean ± SEM) of non-athletes, long distance runners and competitive swimmers, who swam about 90 m with and without breath-holding at 07h30 (arrows). Experimental conditions and symbols are the same as in Fig. 6.

Results

Appendix 3, Tables 2-2 to 2-10

The water temperature at the time of the exercise varied between 17,5° and 24,5°C (mean 21°C), and the subjects swam a total distance of about 90 m on each occasion.

Early morning blood lactate levels were similar in all subjects. The mean concentrations rose approximately three-fold during exercise in the non-athletes and runners, but remained unchanged in the swimmers. There was no significant difference between the lactate levels after underwater swimming and those after aerobic exercise in any group. The lactate levels during the remainder of the day did not differ significantly between any of the groups, nor did they deviate significantly from control day levels (Fig. 6).

Control day blood ketone body (acetoacetate + 3-hydroxybutyrate)

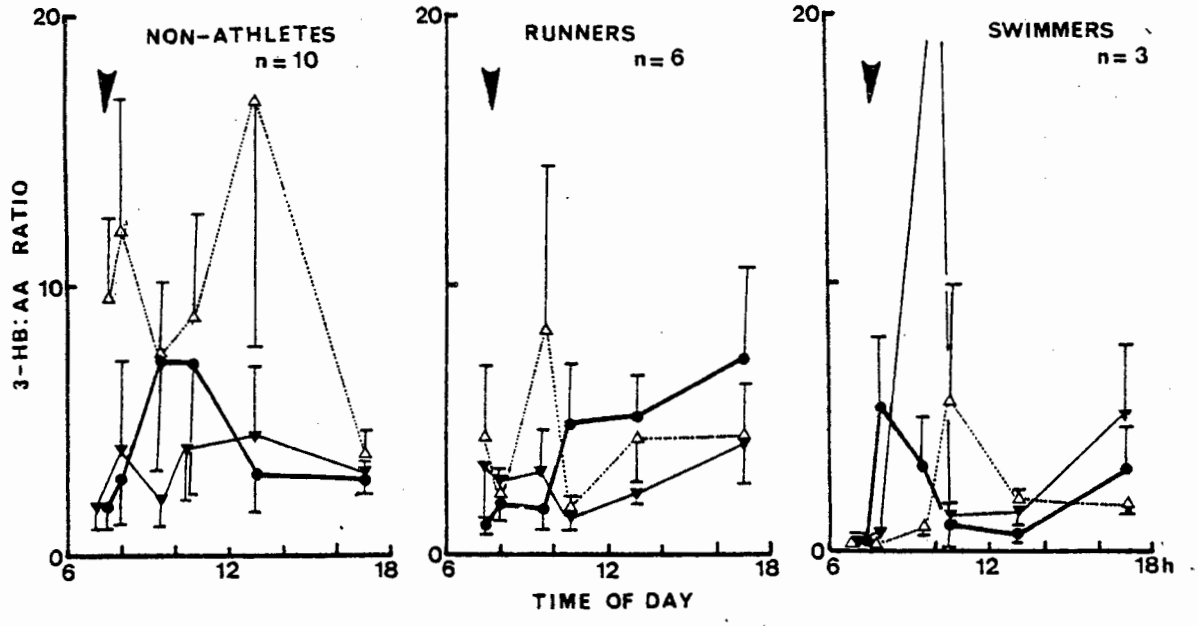

Fig. 8. The daily variations in the blood 3-hydroxybutyrate:acetoacetate (3-HB:AA) ratio (mean ± SEM) of non-athletes, long distance runners and competitive swimmers, who swam about 90 m with and without breath-holding at 07h30 (arrows). Experimental conditions and symbols are the same as in Fig. 6.

concentrations did not differ significantly from those described in Chapter 1. The mean blood ketone body concentrations on exercise days were all slightly higher than on control days, but these differences were not statistically significant. In none of the groups did blood ketone body concentrations after swimming with breath-holding differ significantly from those after swimming without breath-holding (Fig. 7).

The 3-hydroxybutyrate/acetoacetate molar ratio in the blood showed very wide, apparently random, variations which did not appear to be related to exercise training, type of exercise performed, or time of day (Fig. 8).

Discussion

Land mammals, including man, experience bradycardia and a marked reduction in the blood flow to the limbs and skeletal muscles during a dive (13,148,160,209). The mean reduction in the blood flow to the forearm in a resting man on submersion of the

face in water has been found by various investigators to be 15,5% (335), 20% (53), 29% (59), 30% (84), 35% (258), 45% (83), and in some cases 100% (83). There have been no measurements of blood flow to exercising muscles during a dive in man, but indirect evidence would indicate that the circulation remains severely restricted, as in natural divers such as the seal and the duck (84, 148,362). Thus Scholander et al. (297) found that the blood lactate concentration remained unchanged during vigorous underwater swimming in Australian pearl divers, but as soon as the subjects surfaced, their blood lactate concentrations rose acutely, reaching levels similar to those described here, presumably because the blood flow to the limbs had been restored and the accumulated products of anaerobic metabolism were flushed out. Such anaerobic exercise failed to produce a noticeable rise in the blood ketone body concentrations of our subjects.

Not only did blood ketone body concentrations after swimming not rise above normal (highest individual post-exercise concentration was 0,521 mmoles/l at 17h00, in a runner), but the mean levels at 1½ hours after exercise were all lower than at the time of the swim, and at 3 hours were no higher than they had been during exercise. There was therefore no 'Post-Exercise Ketosis' in any sense of the word, in these experiments, and the question of whether it is muscle glycogen depletion during submaximal exercise which predisposes untrained subjects to develop post-exercise ketosis, remains unanswered.

It is possible that the exercise did not last long enough (about 2 minutes of actual swimming time) for ketosis to occur. Most investigators used exercise lasting 1 hour or more to study post-exercise ketosis, but Johnson et al. (61,271) successfully demonstrated a rise in blood ketone body concentration after only 20 minutes of exercise. Fifteen minutes of exercise at 72 W was found by Neufeld et al. (247) to cause a significant elevation of the blood ketone body concentration, and Łukawaska et al. (213) found that the regular Harvard Step Test, which lasts for 5 minutes, produced a significant rise in the immediate post-exercise blood ketone body

concentration. Preti's (265) subject's urinary acetone excretion rose markedly after running up and down stairs, which presumably produced exhaustion within 2 to 3 minutes. Post-exercise ketosis following short bouts of intense exercise would nevertheless appear to be rare, and the results of the present study probably conform to the rule rather than to the exception.

CHAPTER 4

THE EFFECT OF WORK LOAD AND DURATION ON POST-EXERCISE BLOOD KETONE BODY CONCENTRATIONS OF NON-ATHLETES AND ATHLETES

Courtice and Douglas (69) declared that the intensity of post-exercise ketonuria was proportional to the total oxygen consumption ('total work done') during exercise. This conclusion was based on the finding that approximately the same amount of ketones appeared in the urine after a 19 km walk at 5,6 km/h as after a 16 km walk at 7,2 km/h; and that the ketonuria was reduced after a 16 km walk at 5,6 km/h. No other attempts have since been made to study the relationship between post-exercise ketosis and the intensity or duration of work.

Information of this kind would, however, contribute to the understanding of what causes post-exercise ketosis since Saltin and Karlsson (285) have shown that exhaustion after exercise at work loads demanding more than 90% of the subject's maximum rate of oxygen consumption occurred when there was still a lot of glycogen left in the muscles; whereas exhaustion after loads demanding less than 70% of the subject's maximum rate of oxygen consumption, occurred only when the muscles were depleted of glycogen (141,145,285,321).

Therefore, if the glycogen content of the muscles at the end of exercise is one of the main determinants of post-exercise ketosis, it would be expected that brief intense exercise would have less effect on the blood ketone body concentration than prolonged moderate exercise.

Methods

Twenty-three healthy young male students of physiology were studied. None of the subjects had been chronically ill, had a change in body mass or had taken hormones during the previous year. Participation was voluntary and each subject was informed of the intentions and risks of the experiments.

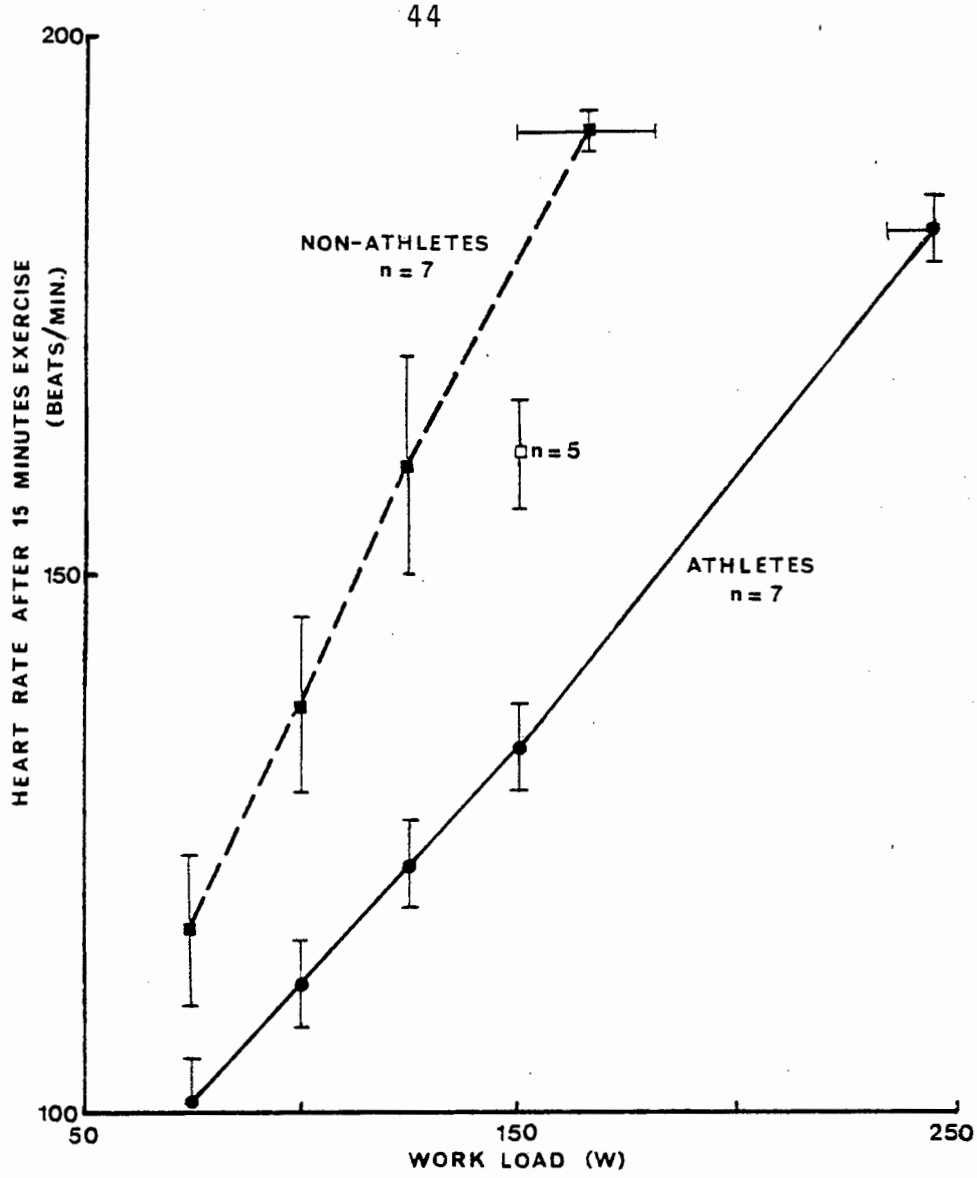

Fig. 9. Heart rate (mean ± SEM) of non-athletes and athletes during the last minute of 15 minute cycling on a bicycle ergometer at different work loads. Maximal work loads were determined for each subject by extrapolation of the plot of his heart rate during submaximal exercise, against work load, to predict tne load which was expected to produce a heart rate of 195 beats/minute after 15 minutes.

Exercise was performed on an electrically braked Lanooy bicycle ergometer, which had recently been calibrated with a device described by Cumming and Alexander (71). All experiments were carried out in the early morning and at room air temperatures of between 18° - 22,5°C, to avoid differences in blood ketone body concentrations arising from circadian rhythms and variations in environmental temperature. Heart rates were recorded electrocardiographically at 15 minute intervals during exercise.

All subjects fasted from the previous evening until the end of the observations at 17h00, consuming only energy-free beverages, but performing their normal daily activities. To minimize any

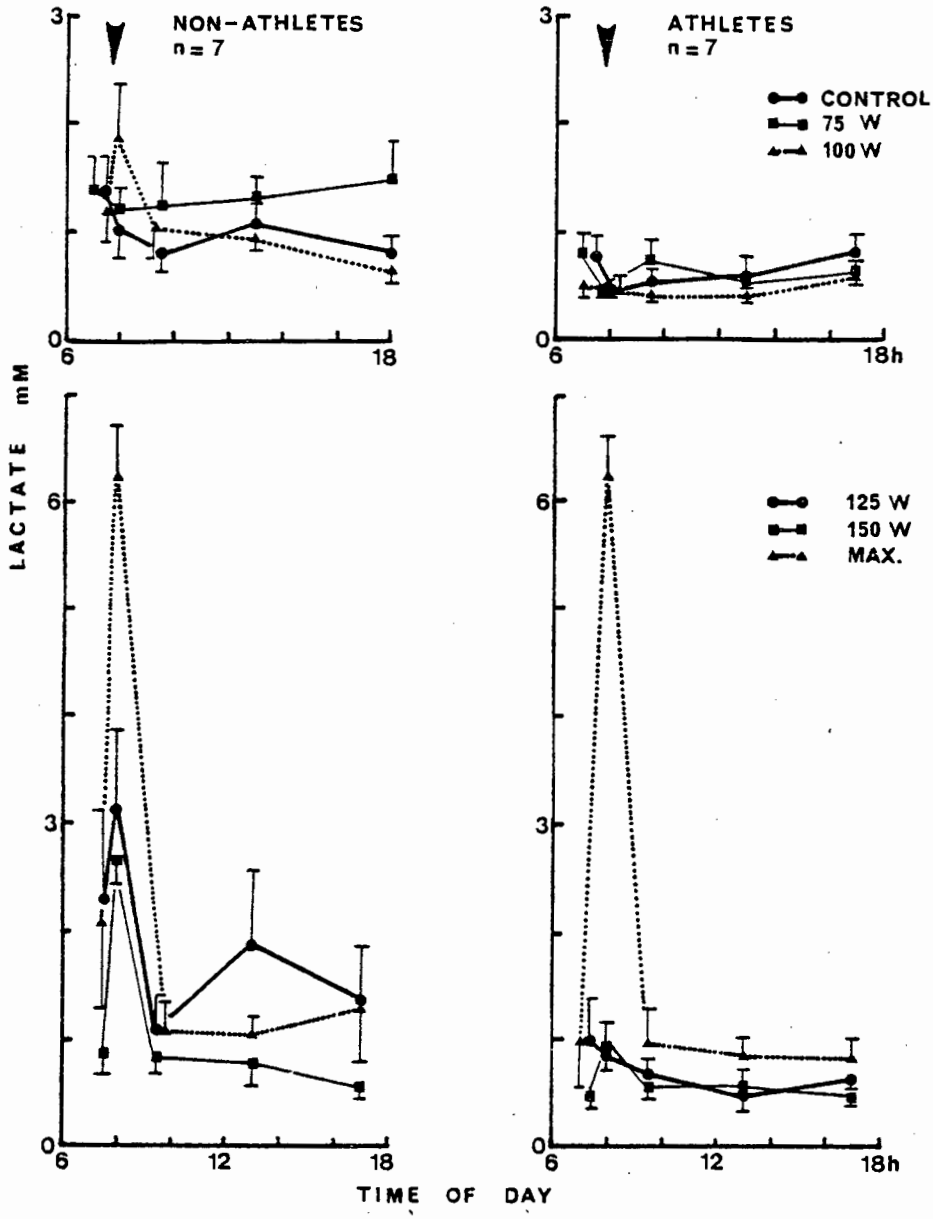

Fig. 10. The daily blood lactate concentrations (mean ± SEM) of 7 non-athletes and 7 athletes who cycled on a bicycle ergometer for 15 minutes at 07h30 (arrows), at different work loads. The last meal was eaten during the previous evening. Subjects performed their normal daily activities, but ate no food during the observation period.

intertest variations that might occur in these activities, each subject was studied always on the same day of successive weeks. This would also avoid the possibility of the subjects becoming trained by the exercise. None of the subjects smoked on experimental days.

All results were compared with the same observations made during a day on which the subjects fasted but did not exercise in the early morning (control days).

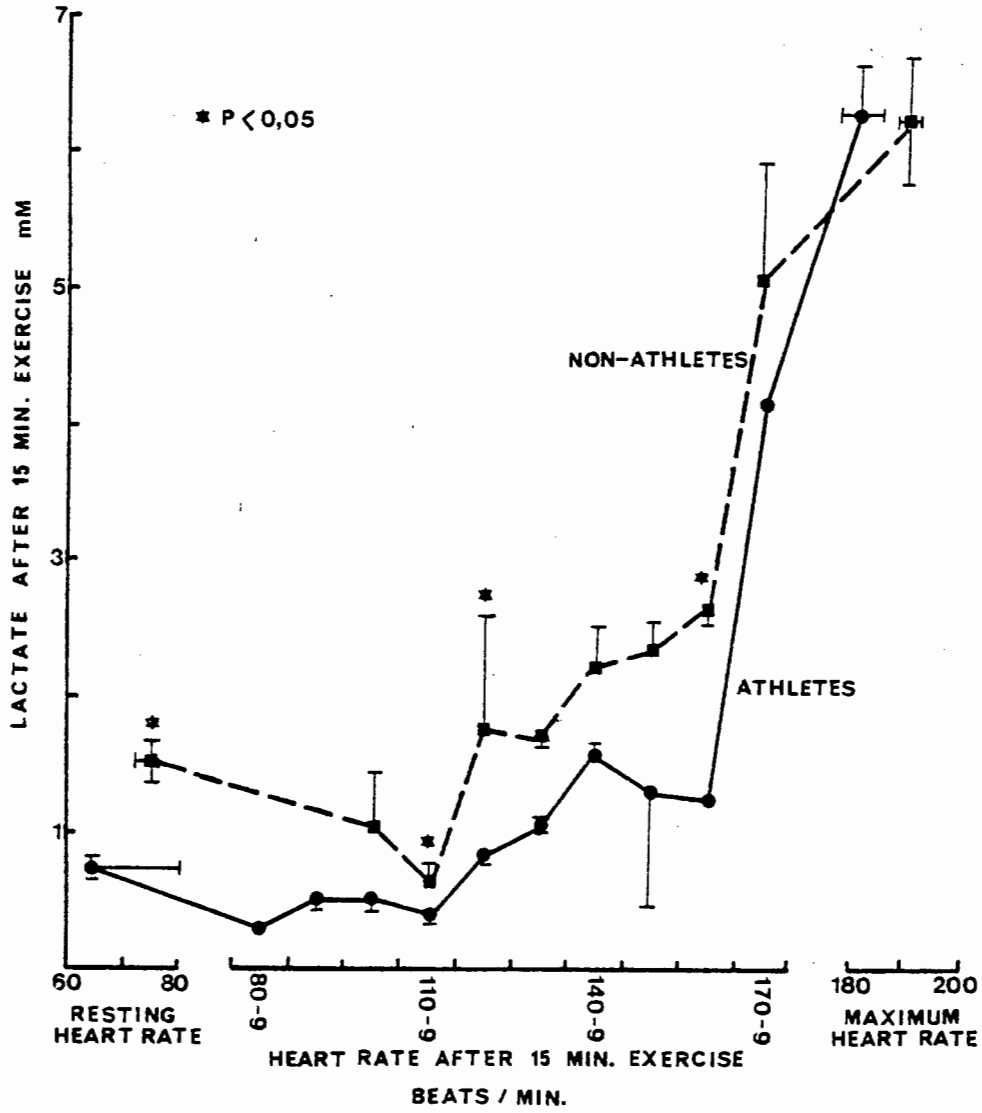

Fig. 11. The blood lactate concentrations (mean ± SEM) of 7 non-athletes and 7 athletes at rest and after 15 minutes of submaximal and maximal exercise. Experimental conditions are the same as in Fig. 10.

A. Intensity of Exercise

The influence of varying intensities of exercise on daily blood ketone body concentrations was studied in 14 subjects, 7 of whom did not take part in any regular sporting activity, while the remaining 7 ran at least 5 km on not less than 4 days in the week. Their ages, heights and masses were as follows:

			Age (years)	Height (cm)	Mass (kg)
Non-athletes	n = 7	mean	19	177	69
		range	19	172 - 184	60 - 76
Athletes	n = 7	mean	19	180	73
		range	18 - 23	176 - 188	71 - 86

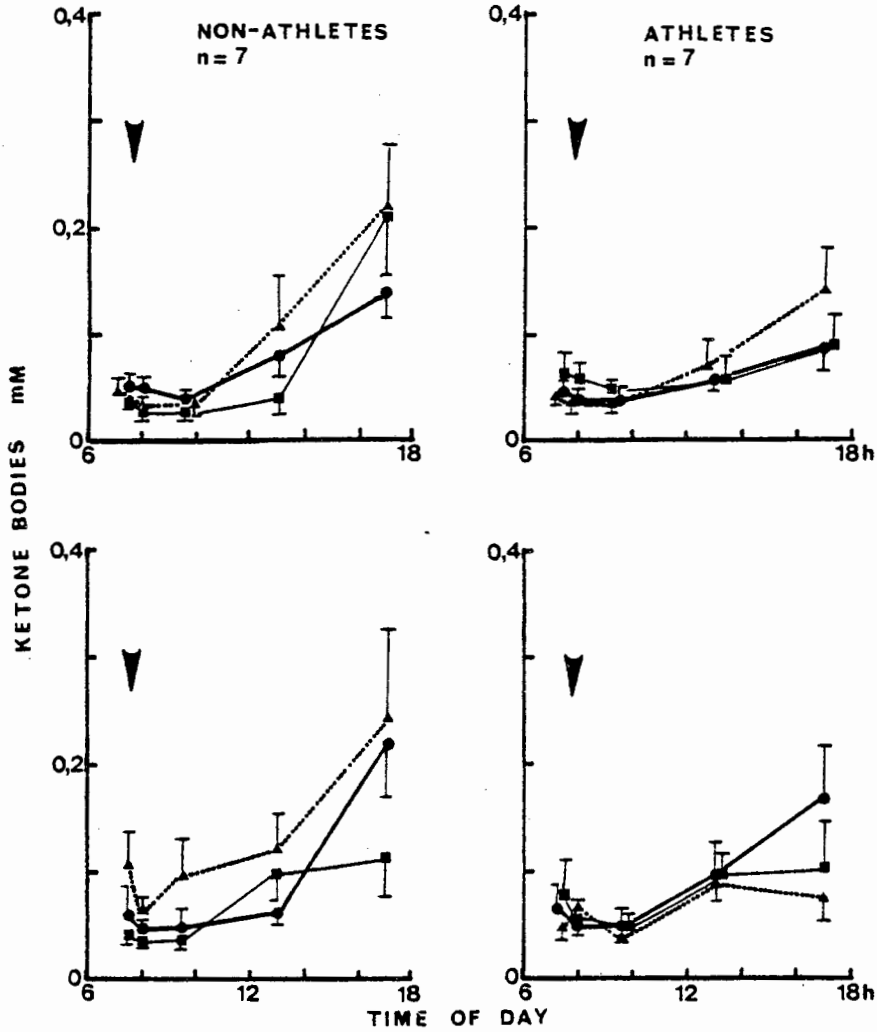

Fig. 12. The daily blood ketone body concentrations (mean ± SEM) of non-athletes and athletes who cycled at different work loads for 15 minutes at 07h30 (arrows) after eating their last meal during the previous evening. Experimental conditions and symbols are the same as in Fig. 10.

There were five test days and a control day. Exercise was performed at 07h30 for 15 minutes at a constant work load. The work load was different (75 W, 100 W, 125 W or 150 W, selected in random order) on each of 4 of the test days. The work load on the 5th test day was determined for each subject by extrapolation from a plot of his heart rate during the last minute of each submaximal exercise against work load, to predict the maximum load he would be able to endure for 15 minutes (i.e. the work load expected to produce a heart rate of 195 beats per minute) (17).

Blood was taken from an arm vein at 07h30, 07h45, 09h30, 13h00 and at 17h00 for determination of the L-lactate,

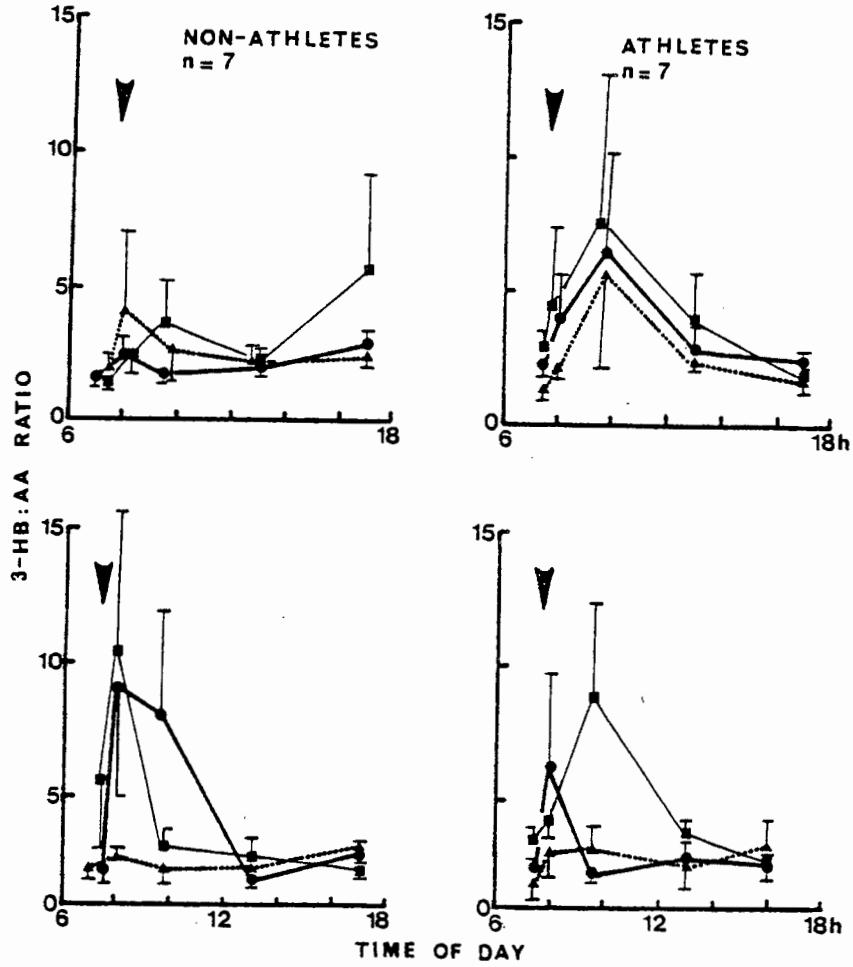

Fig. 13. The variations in the blood 3-hydroxybutyrate:acetoacetate (3-HB:AA) ratio (mean ± SEM) of non-athletes and athletes who cycled at different work loads for 15 minutes at 07h30 (arrows), after eating their last meal during the previous evening. Experimental conditions and symbols are the same as in Fig. 10.

acetoacetate and D-3-hydroxybutyrate concentrations.

B. Duration of Exercise

The influence of duration of exercise on daily ketone body concentrations was investigated in 9 subjects, 6 of whom did not participate in any regular sporting activity, and 3 of whom ran at least 5 km on not less than 4 days in the week. Their ages, heights and masses were as follows:

			Age (years)	Height (cm)	Mass (kg)
Non-athletes	n = 6	mean	19	176	67
		range	18 - 19	169 - 183	58 - 91
Athletes	n = 3	mean	19	184	81
		range	18 - 20	177 - 188	75 - 86

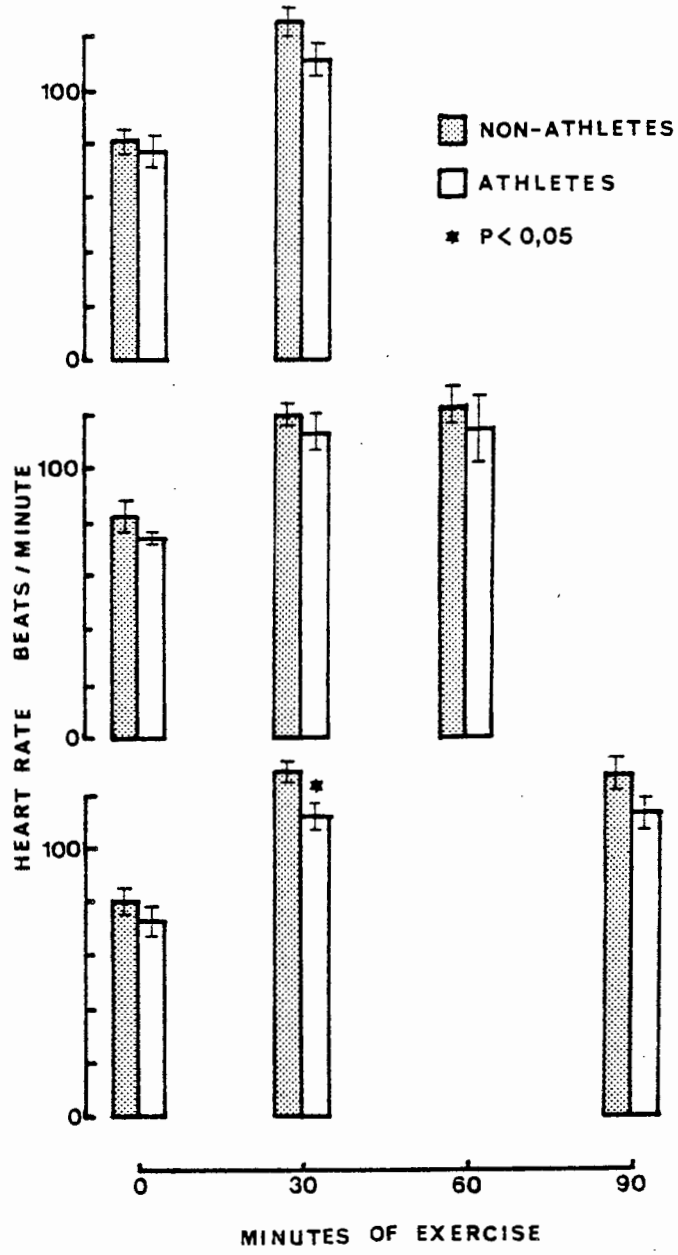

Fig. 14. The heart rate (mean ± SEM) of 6 non-athletes and 3 athletes before and during 30 (top), 60 (middle), and 90 (bottom) minutes of exercise on a bicycle ergometer. The non-athletes worked at 75 W and the athletes at 100 W.

There were 3 test days and a control day. Exercise was performed for 30, 60 or 90 minutes (in random order), always ending at 08h00. The untrained subjects exercised at 75 W on each occasion and the trained subjects at 100 W. These loads were chosen on the basis of the results of Part A (Fig. 9), so that both groups would be exercising at approximately the same heart rates.

Blood was taken before exercise, and at 08h00, 09h30, 13h00 and 17h00 for analysis of the L-lactate, acetoacetate and D-3-hydroxybutyrate concentrations.

Results

A. Appendix 3. Tables 3-2 to 3-13

The mean heart rates of the untrained subjects at rest and at all work loads were significantly higher than those of the trained subjects ($P < 0,05$ to $P < 0,0025$) (Fig. 9). At the predicted maximum work loads, the mean heart rate of the untrained subjects was 191 ($\pm$ SD 6) beats per minute, and that of the trained subjects 182 ($\pm$ SD 7) beats per minute. This difference is statistically significant ($P < 0,025$).

The mean pre-exercise blood lactate concentration was significantly higher in the non-athletes than in the athletes ($P < 0,0005$). Blood lactate levels were also higher in the non-athletes than in the athletes after 15 minutes of exercise at all equivalent submaximal heart rates (reaching statistical significance, $P < 0,05$, whenever there were more than 4 results, for one or other group, at a particular heart rate level) (Fig. 11). The difference between the mean blood lactate concentrations of the two groups after maximal exercise was not significant (Figs. 10 and 11).

There were no statistical differences, in either group, between the total ketone body (acetoacetate + 3-hydroxybutyrate) concentrations in the blood on test days and control days ($P > 0,10$). There were also no significant differences between the results of the two groups (Fig. 12) All ketone body concentrations found in this study were well within the normal range, as described in Chapter 2.

Exercise was associated with a significant rise of the blood 3-hydroxybutyrate/acetoacetate ratio in both non-athletes and athletes after all work loads ($P < 0,02$ - 2 tailed paired t test), but a similar rise was also found to occur between 07h30 and 07h45 on control days (Fig. 13). The extent of the rise in the 3-hydroxybutyrate/acetoacetate ratio after exercise did not appear to be correlated with the intensity of the exercise.

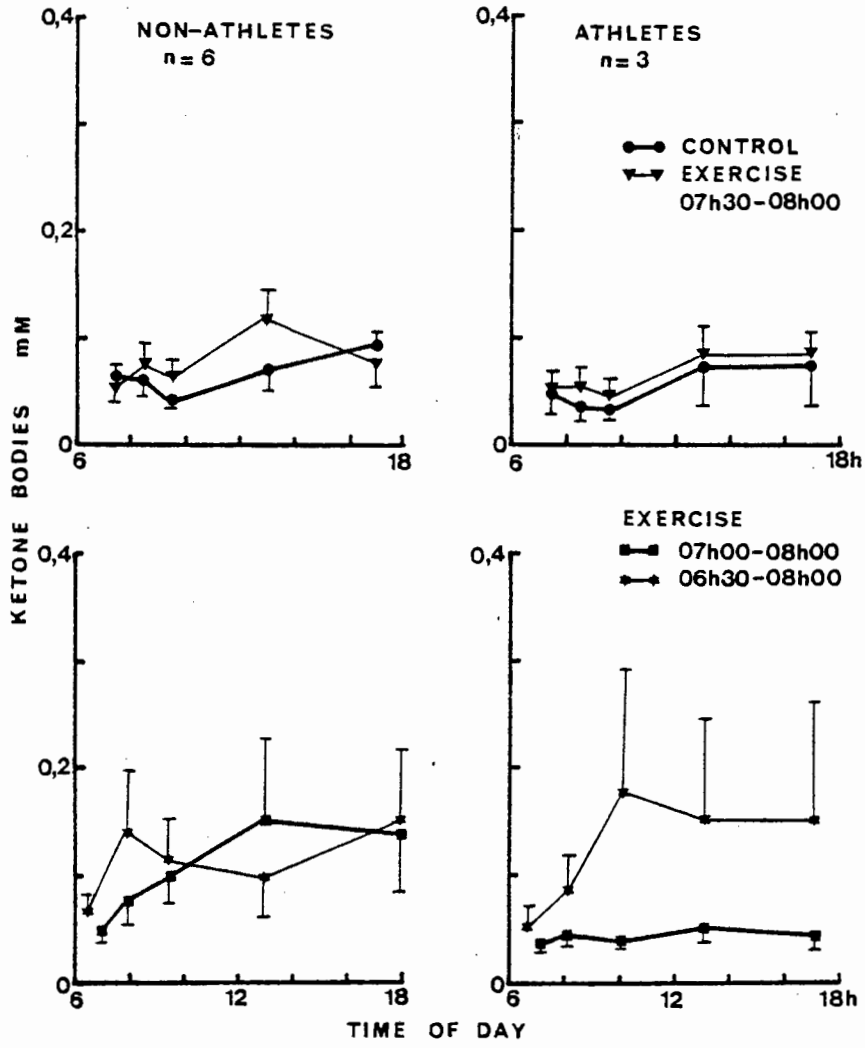

Fig. 15. The daily blood ketone body concentrations (mean ± SEM) of non-athletes and athletes who cycled for 30, 60 and 90 minutes in the morning. Exercise always ended at 08h00. The non-athletes worked at 75 W and the athletes at 100 W, so that their exercising heart rates would be approximately the same (see Fig. 14). The last meal was eaten during the previous evening.

B. Appendix 3. Tables 4-2 to 4-9.

The mean pre-exercise heart rate of the non-athletes (81 ± SD 11 beats/minute) was higher than that of the athletes (75 ± SD 8 beats/minute) ($P < 0,10$). At the end of the exercises the mean heart rate of the non-athletes was 125 (± SD 13) beats per minute, and that of the athletes 112 (± SD 11) beats per minute. This difference is statistically significant ($P < 0,025$ - 2 tail t test) (Fig. 14).

Blood lactate concentrations after exercise were not significantly different from those before exercise, in either group (Fig. 16). The difference between the resting blood

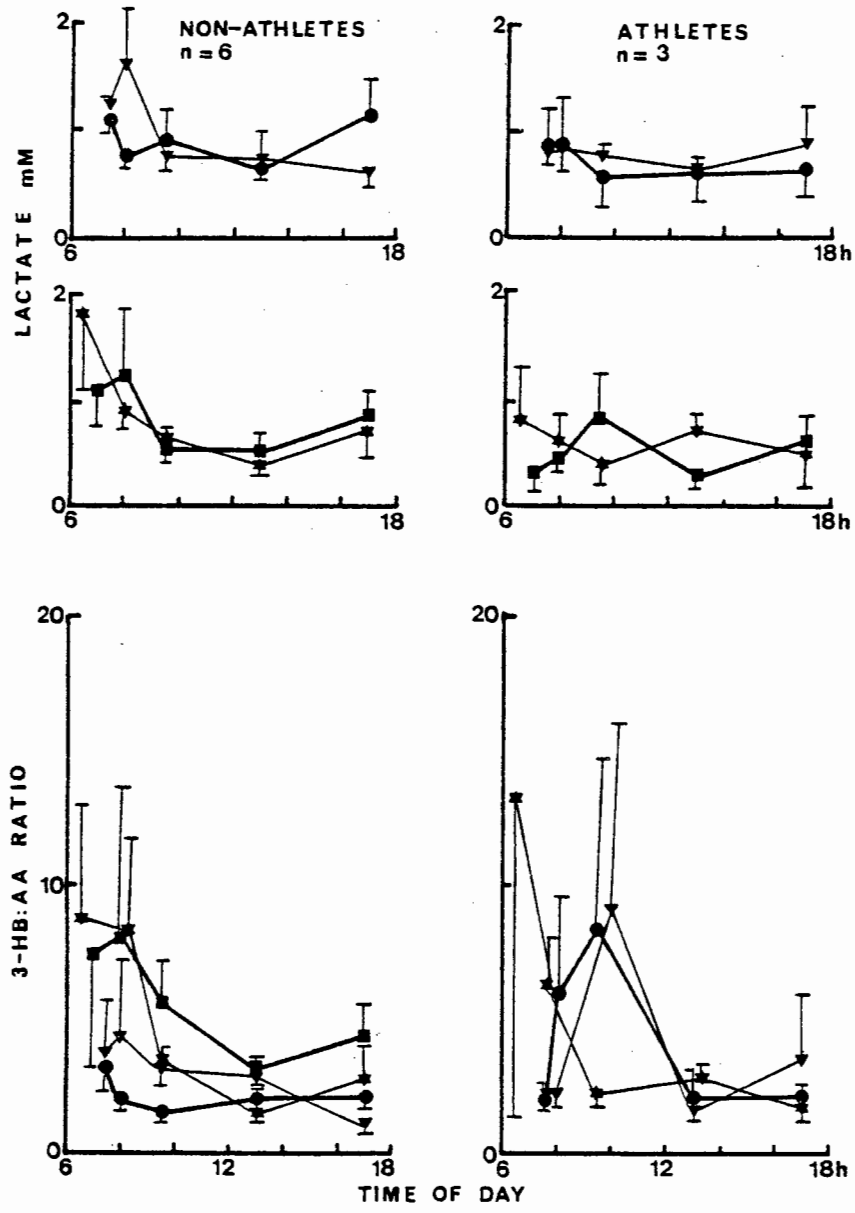

Fig. 16. The daily blood lactate concentrations and blood 3-hydroxybutyrate:acetoacetate (3-HB:AA) ratios (mean ± SEM) of non-athletes and athletes who cycled for 30, 60 and 90 minutes at constant work load in the morning. Experimental conditions and symbols are the same as in Fig. 15.

lactate concentrations of the trained and untrained subjects was statistically significant ($P < 0,05$), but those immediately after 30, 60 and 90 minutes of exercise were not ($P < 0,10$).

Mean post-exercise blood ketone body concentrations did not, in either group, differ significantly from control day values and there were no statistical differences between the results of the trained and the untrained groups (Fig. 15). The highest individual blood ketone body concentration was 0,431 mmoles/l, which occurred in a non-athlete at

17h00, on the day that he had done 1½ hours of exercise.

The 3-hydroxybutyrate/acetoacetate ratio in the blood displayed wide, apparently random variations which did not correlate with duration of exercise, athletic training or with the time ofday (Fig. 16).

Discussion

The maximum blood lactate levels after 15 minutes of exercise in our non-athletes and athletes were similar (i.e. about 6 mmoles/l) to the lactate concentrations found by Rennie, Jennett and Johnson (271) in untrained and trained cyclists after 20 minutes of exercise, but our subjects did not develop post-exercise ketosis, whereas Rennie's untrained subjects did: blood ketones rose from 0,05 mmoles/l before exercise to about 0,18 mmoles/l at 150 minutes after exercise. The mean heart rate (191 beats/minute) and work load (171 W), at which our non-athletic subjects' blood lactate concentrations rose to 6,2 mmoles/l, were higher than those at which Rennie's untrained subjects exercised (160 beats/minute; 116 W). This appears not to be due to greater athletic fitness in our subjects, as their mean heart rate at 125 W (the load closest to the 116 W used in Rennie's study) was also 160 (± SD 26) beats/minute. Furthermore, McGuinness and Sloan (237) found in 1971 that the fitness indices (as estimated by the Harvard Step Test) of students in Scotland and South Africa were about the same. Rennie's cyclists and our runners had similar heart rates during exercise (about 182 beats/minute), but our runners did more work (245 W as opposed to 175 W), indicating that they were probably more highly trained than their Scottish counterparts. (A heart rate of 182 beats/minute is approximately the maximum which trained runners can achieve during exercise. The maximum heart rate of untrained subjects is higher - about 195 beats/minute, as was found here) (17, 192).

When Johnson's group (61) studied 6 untrained subjects who worked for 20 minutes at 100 W (exercising heart rate of 136 beats/minute; peak blood lactate of 2 mmoles/l), blood ketone

body concentrations rose from about 0,04 to 0,06 mmoles/l in the first 20 minutes after exercise and then levelled off. Teräslinna and MacLeod (322) were, on the other hand, unable to find raised blood ketone body concentrations in three untrained subjects after 10 minutes of exercise at work loads ranging from 120 W to 240 W. On two other occasions that post-exercise ketosis was studied after 15 and 20 minutes of exercise (at 75 W and at 50 W respectively), blood ketone bodies did increase, but the exercise had been preceded by a 'ketogenic diet' or by three days of fasting (25, 247).

There was no post-exercise ketosis when our subjects exercised for periods of up to 90 minutes at 75 W (non-athletes) or 100 W (athletes). There was not even a trend towards higher post-exercise blood ketone body concentrations with increasing duration of exercise. When Corbett, Johnson, Krebs, Walton and Williamson (68) studied three untrained subjects who ran for 90 minutes at about 11 km/h, individual blood ketone body concentrations rose to 2,30, 2,35 and 2,65 mmoles/l at 1½ hours after exercise; at that time our highest individual value was only 0,403 mmoles/l, and that occurred in a trained athlete. The highest individual reading at 1½ hours after exercise amongst our non-athletes was 0,297 mmoles/l. In most of Johnson's subsequent reports (166,167,169,173), post-exercise ketosis after 90 minutes of running was less pronounced than in the first report (68) (see Appendix 1), but the average ketone body concentrations at 1½ hours after exercise were still always higher (in the untrained subjects) than we have found them to be here.

A possible reason for this difference could be that our subjects worked relatively less hard than Johnson's subjects. The mean heart rate of our non-athletes after 90 minutes of exercise was only 127 ($\pm$ SD 14) beats/minute, compared with 160 - 165 beats/minute in Johnson's subjects (166). Winder's (356) untrained subjects also had a mean exercising heart rate of 160 beats/minute during their 90 minutes of exercise at 140 W; the mean 1½ hour post-exercise blood 3-hydroxybutyrate

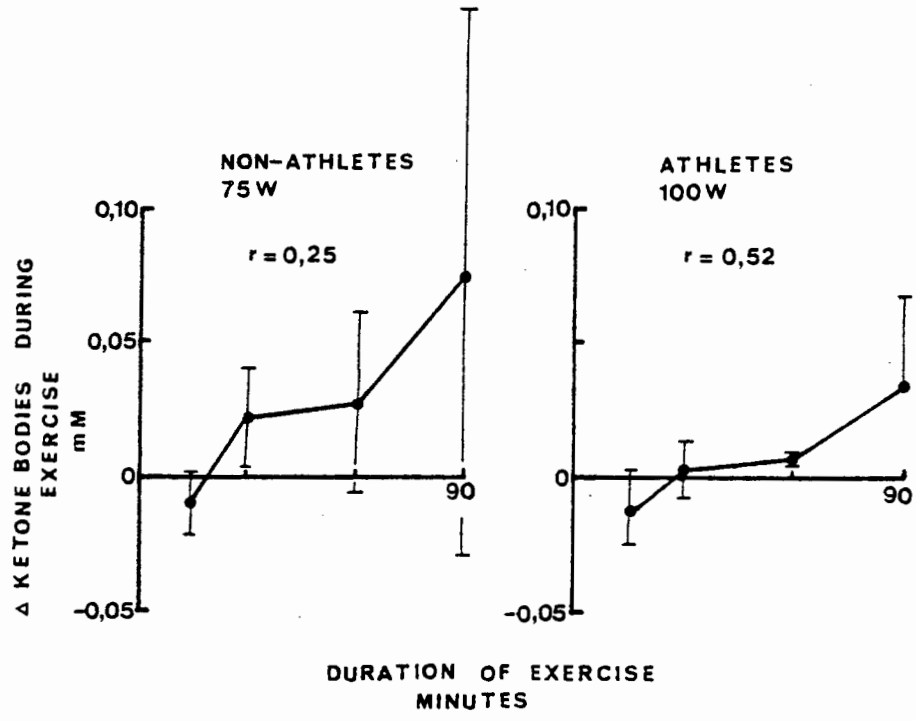

Fig. 17. The changes in the blood ketone body concentrations (mean ± SD) during exercise of constant load but varying duration in non-athletes and athletes. The change is measured from each individual's pre-exercise blood ketone body concentration. The non-athletes worked at 75 W, and the athletes at 100 W, so that their exercising heart rates would be approximately the same (see Fig. 14).

concentration was about 0,42 mmoles/l. Other studies have, however, shown that ketosis can occur after exercise which is both less strenuous and of shorter duration (25,61,85,106,114, 116,171,174,189,213,229,247,265,275,365).

Whether the threshold for the production of post-exercise ketosis in our subjects would have been reached more effectively by prolonging the exercise, or by increasing its intensity, is not known. The questions which these studies were to have answered, must therefore remain in abeyance. The absence of post-exercise ketosis after brief exhausting, and prolonged moderate, exercise could, however, indicate that our subjects were unusually resistant to ketosis, possibly for the same reasons - training excluded - as Johnson's (68,166) athletes

were resistant to ketosis.

Blood ketone body concentrations rose significantly while our subjects were actively exercising for 30 minutes or longer (mean pre-exercise concentration was 0,054 ± SD 0,031 mmoles/l; mean concentration on cessation of exercise was 0,088 ± SD 0,080 mmoles/l. $P < 0,01$ - 2 tailed paired t test). The extent of the rise appeared to be related to the duration of exercise in both non-athletes and athletes (Fig. 17). This latter finding, though not statistically significant in itself ($P > 0,05$), correlates well with the findings of other investigators (46,60,61,82,170,271,273), and probably explains why blood ketone body concentrations have sometimes been found to increase, and at other times to decrease during exercise. During the early part of exercise, blood ketone body concentrations tend to fall, but after 15 - 30 minutes they may level off, and even increase, as they did here and in many of Johnson's investigations. This increase during exercise is frequently continued into the recovery period, to give rise to post-exercise ketosis, but it is not a prerequisite for post-exercise ketosis (e.g. 80,85,174).

CHAPTER 5

THE EFFECT OF AGE ON POST-EXERCISE KETOSIS

As we were unable to demonstrate post-exercise ketosis in young men at the University of Cape Town, a study was undertaken of the effect of age on post-exercise ketosis.

That age might affect post-exercise ketosis is suggested by Åkerblom's (1) failure to find raised blood ketone body concentrations in 15 healthy children, aged 8-14 years, after skiing, running or marching for 45 - 90 minutes, and by Courtice and Douglas' (69) finding that Douglas, aged 52 years, developed post-exercise ketonuria more readily than Courtice, aged 24 years. Also, Johnson's (46,61,68,166,170,173,174,271,272) subjects were, on the whole, older than our subjects, but the differences were not very great.

Moore (243) has shown that maximum athletic performance is achieved at different ages in different sporting events. Speed events such as sprints are usually won by men in their early twenties. Discus and shot-put records are set by slightly older persons, while maximum performance in endurance events, such as Marathon running, is usually not achieved before the age of 30 years. The deterioration which occurs with increasing age is more marked in the sports which depend on strength and speed of muscular contraction (e.g. discus, shot-put and 200 m dash), than in those which depend on stamina. Thus the Marathon race is run as fast by 50 year-olds as by 17 year-olds: at about 90% of the speed of a 30 year-old. Discus and shot-put records at 50 years are about 75% of their value at 30 years.

The biochemistry of these developments is not known, but if post-exercise ketosis is in some way a function of 'athletic fitness' it might be expected to show some age-related trends.

The muscle glycogen content of resting muscle is not affected by age (157).

Methods

Six healthy male members of staff at the University of Cape Town were investigated. None of them took part in any regular sporting activity, had recently observed a change in body mass, or had been treated for any chronic illness during the previous year. Their ages, heights and masses were as follows:

	Age (years)	Height (cm)	Mass (kg)
VS	51	170	65
HW	41	187	66
RL	38 (1)	183	61
EM	38 (2)	186	88
JK	37	178	65
LK	30	186	90

The studies took place during the summer vacation, but the subjects remained indoors for the whole of the observation period (07h30 - 17h00). The air temperature in the laboratory at 08h00 varied between 21,0^{o} and 24,0^{o}C (mean 23,2^{o}C) on the different observation days.

Since one of the subjects in a pilot study (JK, 14-12-77 in Fig. 20) had developed symptoms suggestive of an hypoglycaemic attack one to two hours after exercise, it was considered advisable that all the subjects have a standard breakfast (consisting of two slices of bread with butter, one egg, some cheese and a sugarless beverage) at 06h30 before exercise. Courtice and Douglas (69) reported that such a breakfast did not influence the development of post-exercise ketosis. The subjects fasted for the rest of the day, drinking only energy-free beverages ad libitum. None of the subjects were smokers.

Exercise was performed on a bicycle ergometer at 75 W for 90 minutes (07h30 - 09h00), and blood was taken by venepuncture at 07h30, 08h00, 10h00, 11h00, 12h00 and 17h00 for analysis of the L-lactate, acetoacetate and D-3-hydroxybutyrate concentrations.

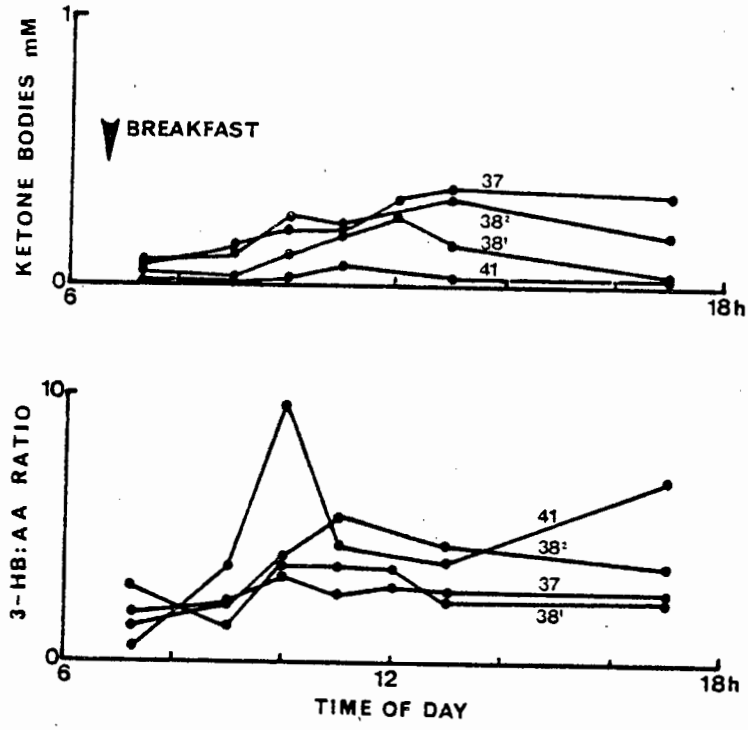

Fig. 18. The daily blood ketone body concentrations and 3-hydroxybutyrate:acetoacetate (3-HB:AA) ratios, of 4 older subjects, who had eaten a standard breakfast at 06h30 (arrow). No food was eaten during the rest of the day. The subjects performed no strenuous exercise during the observation period. The ages (in years) of the subjects are indicated on the graph.

Results

Appendix 3. Table 5-2.

There was great variation in the post-exercise blood ketone body concentrations of these older subjects (Fig. 19). Three of the subjects, aged 37, 38 and 51 years, attained levels which were well above those found in younger subjects after the same exercise. The mean heart rate during exercise, of these three subjects was 131 (± SD 7) beats/minute, and that of the remaining subjects, aged 30, 38 and 41 years, was 128 (± SD 15) beats/minute. This difference is not statistically significant ($P > 0,70$). There was also no significant difference between the mean exercising heart rate of the older subjects and that of the young non-athletes who had performed the same exercise in Chapter 4 ($P > 0,50$).

Post-exercise ketonaemia in these older subjects reached a peak about three hours after exercise, after which it fell in spite of continued fasting. Control day ketone body concentrations were similar to those in younger subjects. (Fig. 18).

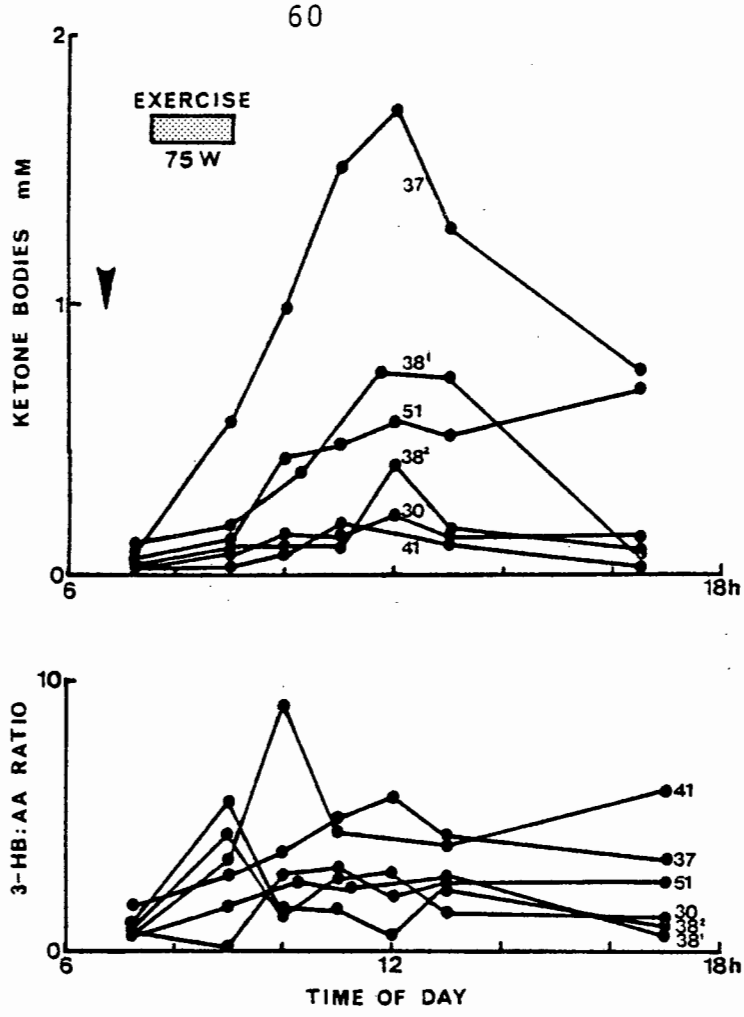

Fig. 19. The daily blood ketone body concentrations and 3-hydroxybutyrate:acetoacetate (3-HB:AA) ratios of 6 older subjects who had cycled for 90 minutes at 75 W from 07h30-09h00. The ages (in years) of the subjects are indicated on the graph. The subjects had all eaten a standard breakfast at 06h30 (arrow). No food was eaten from 07h30-17h00. After exercise the subjects performed sedentary office work till observations were discontinued.

The mean blood lactate concentration in these subjects was 0,64 (± SD 0,26) mmoles/l at rest, and 1,16 (± SD 0,37) mmoles/l after 1½ hours of exercise. This difference is statistically significant ($P < 0,005$).

The 3-hydroxybutyrate/acetoacetate ratio increased during exercise in all but one subject, after which the mean value remained more or less constant for the remainder of the observation period (Fig. 19).

Discussion

Johnson's subjects who developed post-exercise ketosis were aged 17 - 63 years (46,61,68,166,170,173,174,271,272). There are no indications in the different reports, however, that the degree

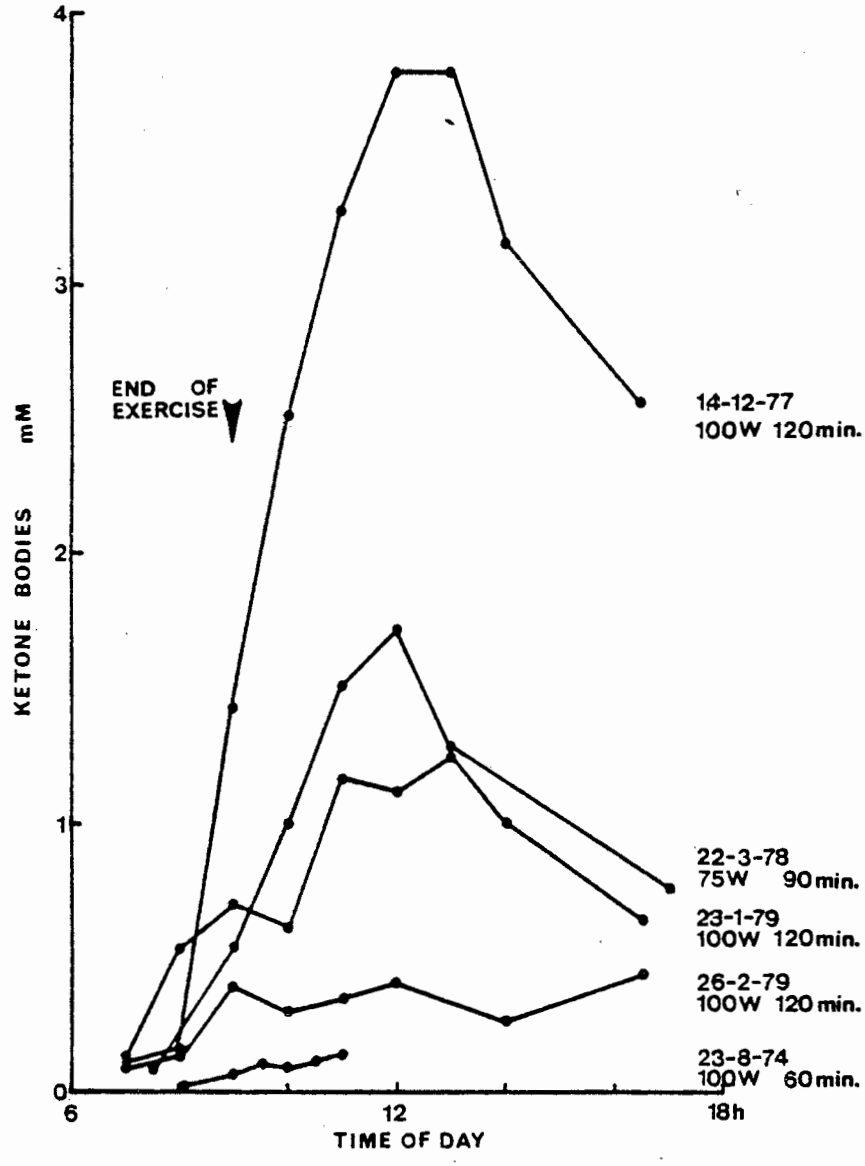

Fig. 20. The daily blood ketone body concentrations of a single subject (JK, born 1940, height 178 cm, mass 65 kg) on different exercise days from 1974-1979. The duration and work load (indicated on the graph) varied on each occasion, but always ended at 09h00 (arrow). No food was eaten during the observation period. Symptoms suggestive of an hypoglycaemic attack occurred between 10h00-11h00 on 14-12-77. These symptoms subsided spontaneously (without taking food), and the subject was able to resume his normal daily activities at 11h30 without feeling faint, sweaty, hungry, or physically exhausted.

of post-exercise ketonaemia was more marked in the older subjects than in the young: in fact, the highest blood ketone body concentrations reported by Johnson et al. (68) were found in three subjects aged 20 - 24 years.

Although we found post-exercise ketonaemia in some older subjects, and not at all in any of our younger subjects, it does not appear that age, as such, was the ketogenic factor, because, in this study, the degree of ketosis bore no relationship to the seniority of the subjects (Fig. 19), nor did it relate at

all to their relative fitness, as judged by their exercising heart rates. Eriksson et al. (85) also found that age (13 - 24 years) had no effect on post-exercise ketosis.

There is a possibility that the altered experimental conditions, under which the older subjects were studied, provided the ketogenic stimulus: the older subjects all had breakfast on the test day, exercised at slightly higher environmental air temperatures (21,0° - 24,0°C compared with 18,0° - 22,4°C) and were on the whole less active during the remainder of the observation period than the younger subjects, who attended classes and practicals in different parts of the Medical School Campus, and therefore did a fair amount of walking hither and thither.

The first two factors would presumably have had an antiketogenic effect during recovery. The effect of 'basal' physical activity on post-exercise ketosis has not been studied, but when subjects lay down for two, and six hours after exercise in Forssner's (106), and Passmore et al.'s (261) experiments respectively, the post-exercise ketonuria was amongst the highest that have been reported. Whatever the combined effects of these conditions were, however, they were the same for all the participants of this study, yet widely divergent individual results were obtained.

That this individual variation was not necessarily due to an age-related expression of genetic polymorphism, is shown by the results of the blood ketone body determinations after exercise in a single individual (JK) from 1974 to 1979 (Fig. 20). These tests were often undertaken as parts of pilot studies, and were therefore not always conducted under identical conditions: the exercises on 23-8-74 and 14-12-77 were carried out in the post-absorptive state (last meal during the previous evening), while the others were all preceded by breakfast at 06h30. In all cases the subject fasted from the beginning of exercise till the end of observations. The indoor air temperatures were not always recorded but the outside air temperatures (provided by the Weather Office, D.F. Malan Airport) were as follows:

	Outside Air Temperature °C	
	08h00	14h00
23-8-74	10,5	15,2
14-12-77	19,0	21,7
22-3-78	17,1	26,7
23-1-79	21,6	37,1
26-2-79	18,6	23,2

The very wide variations in this subject's response were not associated with any known changes in his health, daily habits or in his food intake. His body mass remained constant throughout the observation period. His subjective experience of those work loads which produced post-exercise ketosis, did not differ from the sense of exertion produced by those that did not; but at about 1 to 2 hours after the exercise on 14-12-77 there was suddenly, and quite unexpectedly, a feeling of faintness, blurring of vision and sweating. The symptoms subsided without treatment, when the blood ketone body concentration reached its peak (3,77 mmoles/l) at about midday, after which the subject was able to resume his normal activities, without feeling hungry, or mentally or physically incapacitated. A tentative diagnosis of post-exercise hypoglycaemia (4,157) was made (in spite of a normal blood sugar level of 4,2 mmoles/l at 11h00), and the precaution of having breakfast before exercise, was taken in all subsequent experiments. Similar symptoms did, nevertheless, recur after a 2 hour run by a highly trained marathon runner (TN in Chapter 6), who had been on a carbohydrate-free diet for 2 days. The symptoms were again associated with rapidly rising blood ketone body concentrations, and subsided when the ketonaemia plateaued at about 3 mmoles/l 3 hours after exercise (Fig. 21).

Further experiments were carried out on JK to find the reasons for some of the variations in his response. These experiments are described in Chapter 6 (B).

CHAPTER 6

DIET AND POST-EXERCISE KETOSIS

Endurance athletes have, over the past decade, frequently resorted to 'glycogen loading' (18,105,206,309) to improve their stamina during a race, after it was shown by various Swedish workers, in 1966 - 1967, that fatigue during prolonged exercise occurred when the glycogen in the muscles was used up (6,38,39,40,82,141,154).

Attempts at increasing the glycogen content of the muscles before a race, soon showed that the highest concentrations were obtained if the glycogen in the muscles was first depleted by training on a carbohydrate-free diet for 2 days ('glycogen stripping') (5). If this was followed by rest and a high carbohydrate diet, the muscle glycogen content would increase to nearly twice the normal value in about 3 - 4 days ('glycogen loading') (5,39,155,157,181). Much publicity has been given to these findings (18,105,206,309), and most competitive long distance runners are now familiar with these procedures, which appear to have definite beneficial effects in some runners (39,181), but not in others (27,303,309).

Rennie and Johnson (175,273) studied athletes on a normal diet and after 'glycogen loading', and found that the blood glucose, lactate and pyruvate concentrations were higher, and the blood glycerol and plasma free fatty acids were lower during exercise, following the glycogen enhancing regimen. Post-exercise ketone body concentrations were lower after glycogen loading than before. These results indicated that the increased glycogen content of the muscles gave rise to greater carbohydrate utilization during exercise.

The purpose of the present study was to investigate the fuel-hormone response to exercise after 'glycogen stripping' (5), and its effect on post-exercise ketosis.

Methods

A. The subjects were two male members of the University of Cape Town Running Club, who ran at least 120 km per week. They were in good health, had not observed a change in their body mass during the previous year, had never had any hormone therapy, and did not smoke. Their ages, heights and masses were:

	Age (years)	Height (cm)	Mass (kg)
TN	29	187	77
DC	24	176	67

Their participation in the investigation was voluntary, and they were fully informed of the procedure to be followed, and of the possible risks.

There were two test days and a control day, on which the subjects ate a standard breakfast (consisting of an egg and bacon, some cheese and a cup of milk) at 06h30. No food was consumed after that, till 16h30, but energy-free beverages were taken ad libitum. The exercise on the two test days consisted of running at 12 - 13 km/h on a level treadmill for 2 hours, from 07h00 to 09h00. They performed sedentary office work during the remainder of the day till 16h30. No strenuous exercise was undertaken on the control day.

Prior to the first test day, the subjects ate their usual mixed diet, which contained about 500 g total carbohydrate per day. On the two days before the second test, they ate a protein-fat diet consisting of meat, fish, eggs, leafy green vegetables and unsweetened dairy products ('glycogen stripping'). The subjects did their normal training during the 48 hours before, but not on, both test- and the control-days.

B. The 37 year-old non-athletic subject (JK, height 178 cm, mass 65 kg) who had the highest blood ketone body

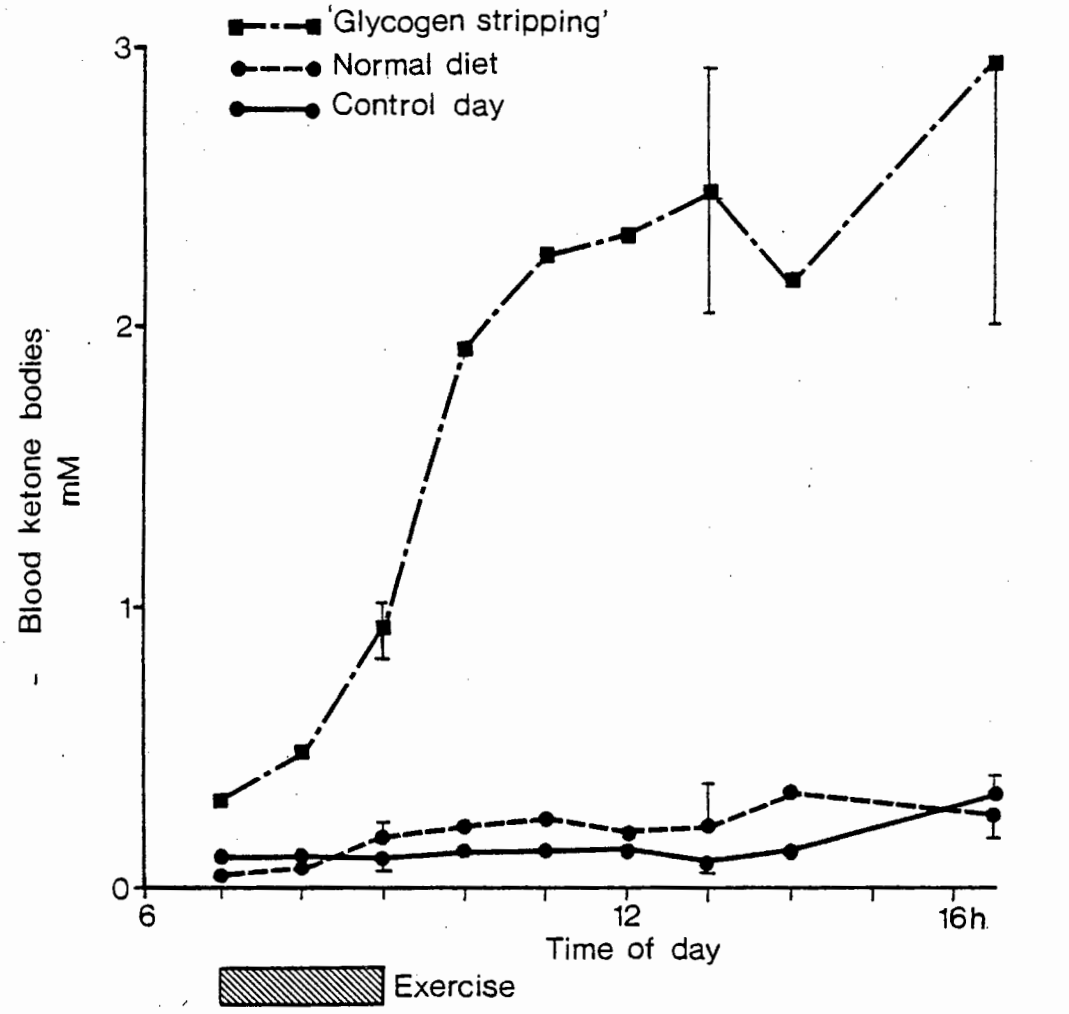

Fig. 21. Mean fasting blood ketone body concentrations in 2 highly trained marathon runners after running at 12-13 km/h for 120 minutes, following a normal diet, and "glycogen-stripping". The "glycogen-stripping" regimen consisted of eating a protein-fat diet for 2 days while continuing normal training. The range of the readings (n=2) is indicated at 09h00, 13h00 and at 16h30. The subjects rested after exercise, and ate no food during the observation period.

concentrations after exercise in the investigation described in Chapter 5, was studied to find out how diet affected his post-exercise ketosis.

There were 6 test days, each a week apart, on which the subject exercised at 100 W on a bicycle ergometer for 2 hours (07h00 to 09h00) after having eaten a standard breakfast as described in Chapter 5. The rest of the day was devoted to laboratory work.

Before the first test day the subject ate his usual diet,

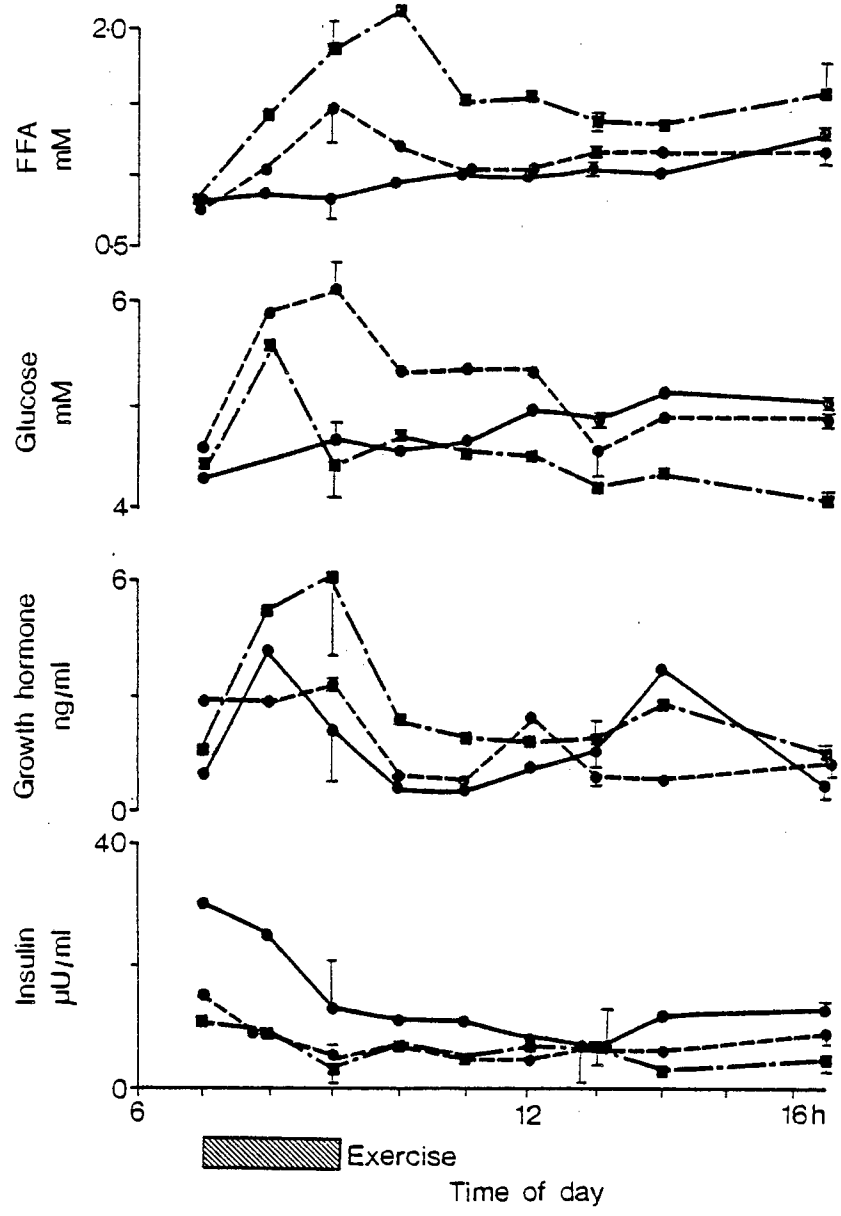

Fig. 22. Mean fasting serum free fatty acid (FFA), glucose, growth hormone and immunoreactive insulin concentrations in 2 marathon runners after running at 12-13 km/h for 120 minutes. Experimental conditions and symbols are the same as in Fig. 21.

which contained approximately 250 g total carbohydrate per day. For 2 days before the second and third tests he added sugar (sucrose) to his tea and coffee, which increased his carbohydrate intake by 60 - 90 g per day; and the fourth and fifth tests were each preceded by 2 days of carbohydrate restriction (total carbohydrate intake of about 80 g per day). The sixth test was carried out after he had returned to his usual diet for a week.

Venous blood was taken at hourly intervals from all three

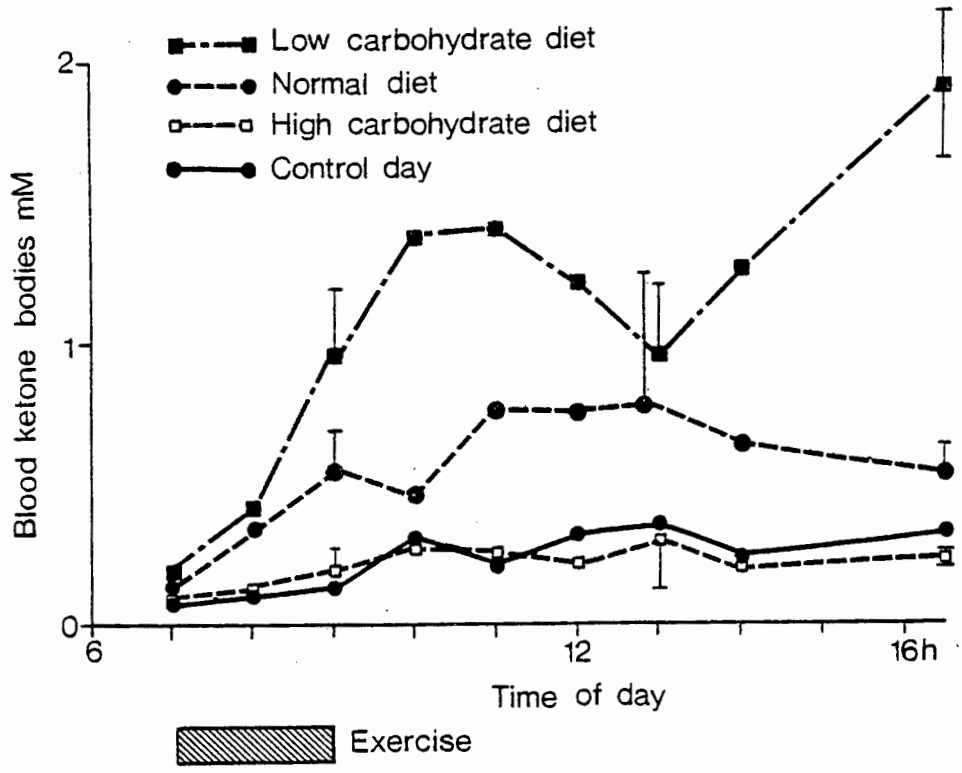

Fig. 23. Mean fasting blood ketone body concentrations in the 37 year-old subject from Fig. 19, after 120 minutes cycling at 100 W, following various dietary regimens on the 2 days preceding the exercise. Each test was done in duplicate and the range of the readings is indicated at 09h00, 13h00 and at 16h30.

subjects (TN, DC and JK) from 07h00 to 14h00 and again at 16h30, for the determination of the acetoacetate, D-3-hydroxybutyrate, glucose, free fatty acid, immunoreactive insulin (IRI) and immunoreactive human growth hormone (HGH) concentrations. The lactate concentrations were not measured.

Results

Appendix 3. Tables 6-2 to 6-8.

Ketone Bodies

Post-exercise blood ketone body (acetoacetate + 3-hydroxybutyrate) concentrations after a normal diet in the long-distance runners did not differ from control day values. Exercise after glycogen stripping caused a marked elevation of the blood ketone body levels (Fig. 21). Unlike the post-exercise ketosis seen in

Chapter 5 (Figs. 19 and 20), however, the ketosis after carbohydrate restriction was biphasic, with an early peak at about 13h00, followed by a dip and a second more sustained rise to 3,88 mmoles/l in TN, and 2,02 mmoles/l in DC, at 16h30.

On a normal diet the post-exercise blood ketone body concentration in the non-athlete JK rose to a single peak at about midday, as it had done before (Figs. 19,20 and 23). The peak ketone body concentrations (1,26 and 0,41 mmoles/l on the first and sixth test days respectively) were, however, lower than after the shorter and less strenuous exercise described in Chapter 5 (1,72 mmoles/l). A low carbohydrate diet produced a biphasic response similar to that observed in the two long distance runners after glycogen stripping. The early peak occurred about 2 - 3 hours after exercise and was followed by a trough and a second rise to 2,18 and 1,64 mmoles/l at 16h30 on the first and second carbohydrate restriction days respectively. After a high carbohydrate diet the blood ketone body concentrations after exercise were indistinguishable from those on the control day (Fig. 23). There seemed to be a tendency over the six-week period that these tests were conducted, for the 11h00 peaks in the post-exercise blood ketone body concentration after the different diets, to converge to a common value (Fig. 25).

Free Fatty Acids

Serum free fatty acid concentrations rose to a peak during exercise in all three subjects, and fell within 1 - 2 hours after exercise to a plateau which lasted as long as the period of observation (Figs. 22 and 24). The concentrations at the end of exercise were inversely related to the previous days' carbohydrate intake. On more-or-less comparable diets, the non-athlete's and the athletes' serum free fatty acid concentrations rose to similar extents during exercise.

Glucose

In the non-athlete the serum glucose concentration was

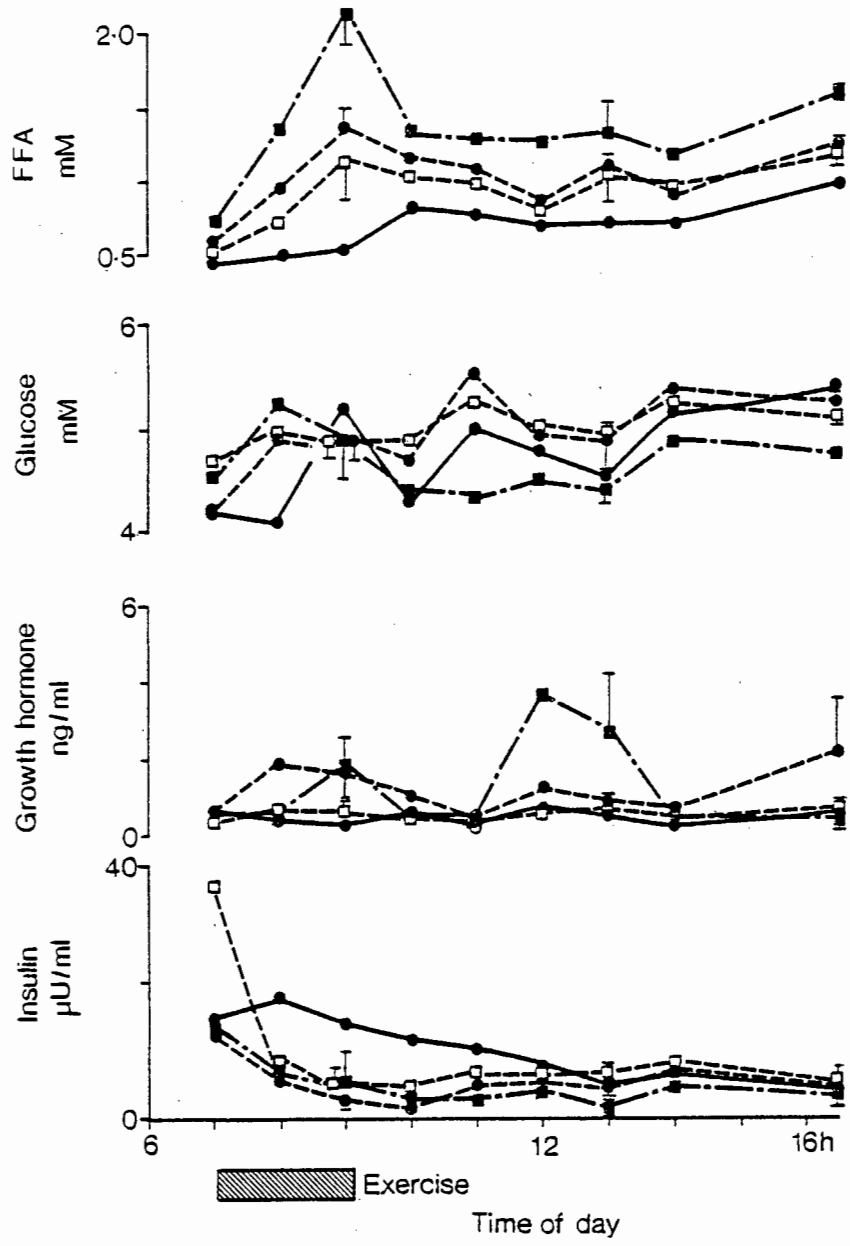

Fig. 24. Mean fasting serum free fatty acid (FFA), glucose, growth hormone and immunoreactive insulin concentrations in the 37 year-old subject from Figs. 19 and 23 after 120 minutes of cycling at 100 W. Experimental conditions and symbols are the same as in Fig. 23.

apparently unaffected by diet or by exercise (Fig. 24). The long-distance runners showed a marked increase in the serum glucose concentration during exercise (Fig. 22), but after glycogen stripping this was followed by a fall before the end of the exercise. This fall continued during the recovery period, to reach levels which were significantly lower than those on the control day at 16h30 ($P < 0,05$; 2-tailed, paired t test).

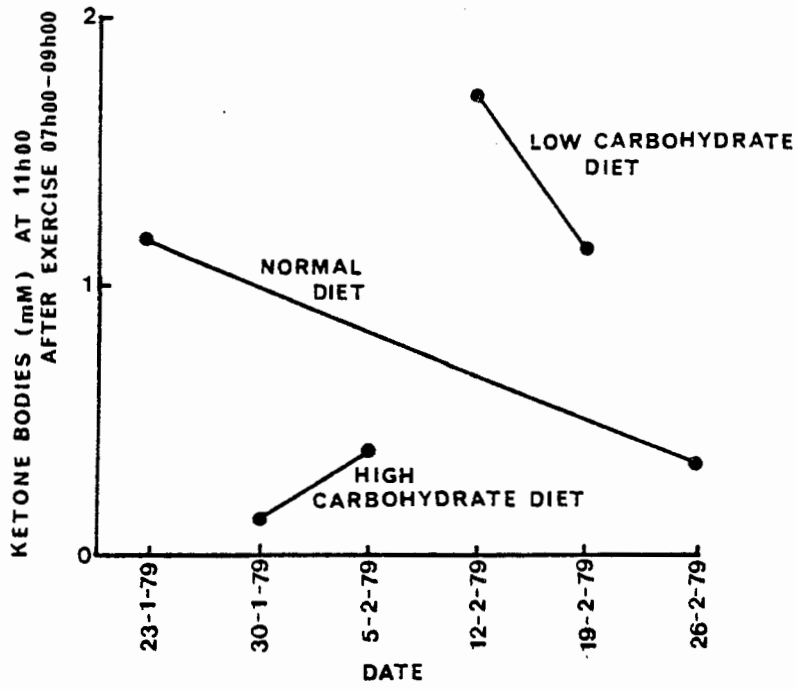

Fig. 25. The blood ketone body concentrations at 11h00 in the 37 year-old subject of Fig. 23 after 120 minutes cycling at 100 W from 07h00-09h00, following various dietary regimens on the 2 days preceding the exercise.

Immunoreactive Insulin (IRI)

Serum IRI concentrations on control days fell gradually from a mean value of 26 µU/ml at 07h00 to 6,5 µU/ml at 13h00 and then remained more or less unchanged till 16h30, in all three subjects. Exercise reduced the serum IRI levels more rapidly, so that a mean value of 6,1 µU/ml was reached at 09h00, after which it remained largely unaltered till 16h30. Diet and physical training had little effect on the IRI levels during and after exercise (Figs. 22 and 24).

Human Growth Hormone (HGH)

The concentration of HGH in the serum rose significantly during exercise ($P < 0,05$; paired t test) in all three subjects, except in the non-athlete after a high carbohydrate diet (Figs. 22 and 24). The highest levels occurred at the end of exercise following glycogen stripping in the athletes (Fig. 22). Pre-exercise levels were regained within an hour of finishing the exercise, and then followed a pattern similar to that on the control day, frequently with a peak in the middle of the day.

Discussion

Diet and the Intensity of Post-exercise Ketosis

On many of the occasions that ketosis has been found to occur after exercise, the subjects had been on a low carbohydrate diet, either for incidental reasons, or because post-exercise ketonuria had failed to develop on a normal mixed diet (14,25,30,69, 106,116,138,229,247,265). Post-exercise ketosis has, on the other hand, never been found to occur after a high carbohydrate diet. There can therefore be no doubt that the composition of the diet is important in determining to what extent the blood ketone body concentration will rise after exercise (14,69,81, 273).

Courtice and Douglas (69) showed that the intensity of an individual's post-exercise ketonuria varied inversely with his carbohydrate intake on the previous day, and they surmised that inter-individual variations in post-exercise ketonuria were probably also due to diet (241). This has, however, never been verified. In fact, when Passmore and Johnson (261) studied ten healthy medical students, aged 20 - 22 years, after they had all been on an identical diet (1,9 MJ protein + 5,7 MJ fat + 5,0 MJ carbohydrate = 12,6 MJ food energy/day) for 2 days, and had then performed the same exercise under similar environmental conditions, the blood and urinary ketone body concentrations varied widely between the subjects. Calculations based on the oxygen consumption, carbon dioxide production and nitrogen excretion during exercise showed that different amounts of protein, carbohydrate and fat had been metabolised by the different subjects to provide the energy for the work they had done. It was these differences in fuel utilization, in particular the proportion of the energy derived from fats during exercise, which appeared to determine the degree of the ketosis afterwards.

Our results confirm Courtice and Douglas' (69) finding that the ketosis after exercise changes with the previous days' carbohydrate consumption (Fig. 23); but they also show, as Passmore and Johnson's (261) results have done, that considerable variation

can occur even if the diet is kept the same (Fig. 25).

Ketosis did not occur when the 2 long distance runners ran for 2 hours after eating their usual diet, but a marked post-exercise ketosis developed when they had eaten almost no carbohydrates for 2 days (Fig. 21). Carbohydrate restriction in subject TN caused the concentration of ketone bodies in the blood to rise from 0,26 mmoles/l before exercise, to 3,22 mmoles/l at 3 hours after exercise. This was similar to the post-exercise ketosis found in JK on 14-12-77 (Fig. 20), and was also associated with sweating and faintness between 10h00 and 11h00 (see Chapter 5). Subject DC did not experience any untoward symptoms after exercise, but the increase in his blood ketone body concentration was smaller than TN's, reaching only 1,86 mmoles/l at 11h00.

These results indicate that trained athletes are susceptible to post-exercise ketosis, when a diet-exercise programme depletes them of glycogen. Forgac, in 1979 (105), warned athletes of this possibility, and advised them not to eat a diet completely devoid of carbohydrates during the stripping phase of the glycogen loading regimen, in order to avoid the "irritability and fatigue" which, she claims, is caused by ketosis. Young (363), writing in "The Complete Marathoner", also mentions that 'acetone breath' can occur after hard training, and that it is a signal that more carbohydrate should be eaten if performance is to be maintained.

Athletes normally probably do not develop post-exercise ketosis when training, or are exercise-tested, because they eat enough carbohydrate to maintain their body glycogen stores in the face of their high daily energy expenditure (74,202,227): our long distance runners ate almost twice as much carbohydrate per day as JK. But when all 3 subjects exercised for 2 hours after eating almost no carbohydrate for 48 hours, the athletes had higher post-exercise blood ketone body concentrations than the non-athlete. This was almost certainly due to the training which the athletes had done while on the low carbohydrate diet. When Passmore and Johnson (261) gave all their subjects the

same standard 12,6 MJ mixed diet per day, the athletes also became more ketotic after exercise than the non-athletes. This diet was presumably adequate for the usual daily activities of normal subjects, but was probably inadequate for the energy needs of athletes engaged in regular sporting activities. In our present study, and in the investigation of Passmore and Johnson (261), the athletes' glycogen stores on experimental days were therefore probably lower than those of the non-athletes, thus pre-disposing the athletes to higher blood ketone body concentration after exercise than the non-athletes.

The higher blood ketone body concentrations in Grollman and Phillips' (129), and in Askew, Dohm and Huston's (14) trained rats than in their untrained controls, immediately after exhausting exercise were probably also due to decreased fuel depots caused by training on an inadequate diet. In fact, Askew et al. (14) noted that their trained rats ate significantly less food than the untrained rats - a phenomenon that is sometimes observed when these animals perform excessively heavy exercise for a long time (227). Unfortunately, Grollman and Phillips (129) do not mention how much their trained and untrained animals ate. On the other hand, the rats in both investigations were all exercised to exhaustion, which presumably meant that their muscle glycogen was depleted, whatever their diet or training had been beforehand. Differences in immediate post-exercise ketonaemia were nevertheless found, not only between trained and untrained animals, but also between animals on different diets (14). This means that ketogenesis is not stimulated directly by the low concentration of glycogen in the muscles, but, more likely, by the glycogen content of the liver, which also varies with diet and exercise, but presumably not in exactly the same manner as muscle glycogen (7,26,40,112,122,147,156,195,275,280).

To what extent it would be possible to eliminate all individual variations in post-exercise ketosis, even between trained and untrained subjects, by means of standardised diets and strictly controlled daily activity is not known. The wide variations seen in a single person's response to exercise, when his daily

activities and diet varied very little over the observation period (Fig. 20), would indicate that differences in post-exercise blood ketone body concentration would continue to occur. It also seems unlikely that the biochemical adaptations to endurance training (82,146,196,354) would be entirely without effect on post-exercise ketosis.

Diet and the form of Post-exercise Ketosis

Carbohydrate restriction increased not only the height of the post-exercise blood ketone body curve, but also changed its shape from being monophasic (Figs. 19 and 20) to being biphasic (Figs. 21 and 23). Forssner (106), and Courtice and Douglas (69), found biphasic patterns when the rate of ketone body excretion in the urine was studied for 9 to 12 hours after exercise. Here too, the subjects had been on a low carbohydrate diet before the exercise.

This suggests that there are probably two ketogenic processes after exercise: an initial, apparently self-limiting one, which may or may not be superimposed on a second, slower process, which is particularly prominent when there has been less carbohydrate in the diet than normal (see Chapter 8). It is not certain whether the two processes can occur independently of one another; most of the apparently monophasic curves in Figures 19 and 20 had not returned to normal when observations were discontinued at 16h30, and could therefore have been followed by a second increase in the blood ketone body concentration during the evening. But one person's blood ketones did fall to normal after a single peak during the third hour of recovery (38 year-old RL; Fig. 19), providing the only known example of post-exercise ketosis which has been followed to completion in a subject on a normal diet.

Effect of Diet and Exercise on the Serum Free Fatty Acids

Serum free fatty acid concentrations have consistently been found to rise in response to prolonged exercise in sedentary adults (28,111,166,170,189,259,274), teenagers (85,251,357),

and in athletes (46,166,171,226,271,272,273,295), as well as in patients with diabetes mellitus (37,134,332), hypopituitarism (28,168,170,375), acromegaly (174) and adrenalectomy (on cortisone replacement therapy) (28). Exercise also increases the concentration of free fatty acids in the serum of rats (48,87, 201). A fall in the serum free fatty acid level has been seen only in exercise of short duration, or during the first 10 - 15 minutes of prolonged exercise (46,60,61,108,134,240,271,344). Maximum concentrations usually occur about 6 to 60 minutes after the exercise has stopped (46,60,108,133,166,251,271,272,356).

Our results are consistent with this general pattern, but show, in addition, that the rate of increase during exercise is dependent on the previous days' diet (Figs. 22 and 24). Similar results were obtained by Williams et al. (344), Kuroshima et al. (201), Rennie et al. (175,273) and Bonen et al. (47), showing that a low carbohydrate diet increases the serum free fatty acid levels during exercise, and a high carbohydrate diet decreases them. Hunter et al. (158) and Ahlborg et al. (8) showed that the free fatty acid levels fall to normal when glucose is given during exercise.

Serum free fatty acid concentrations during exercise were similar in all three of our subjects, but other investigators have found significant differences between the levels in athletes and non-athletes. Johnson's non-athletes usually had higher free fatty acid concentrations during exercise than his athletes (68,166,173,271,272), but not always (171). Winder et al. (356) and Devlin et al. (75) also found that untrained subjects had higher levels than trained subjects during exercise, but Bloom et al. (46) found the opposite. Jones et al. (179) and Karlsson et al. (183) found that training did not affect the free fatty acid concentration during exercise. In view of the differences in dietary habits between athletes and non-athletes, and of the importance of the diet in determining the behaviour of the free fatty acids during exercise, it is likely that these inconsistencies were due simply to the food which each group ate before the exercise.

There was no correlation between the serum free fatty acid and the blood ketone body concentrations of our subjects during the 9½ hour observation period. The apparent parallelism which Johnson et al. (166) had found between these metabolites in their first report, in 1969, was not confirmed by later studies (46,61,171,173,271,272), though the possibility remained that this was due to a lag between the peak concentrations in the blood of the substrate and its product. Rennie et al. (275) showed, however, that an increased serum free fatty acid concentration caused by the administration of corn oil (by stomach tube) and heparin (by subcutaneous injection) to rats immediately before exercise, did not influence post-exercise ketonaemia. Our results confirm that the rise and fall of the ketone bodies after exercise are independent of the serum free fatty acid levels, and that post-exercise ketosis is therefore not a mere by-product of free fatty acid homeostasis during exercise.

Effect of Diet and Exercise on the Blood Sugar

The blood glucose concentration appears to be more stable during exercise than the free fatty acid concentration: many authors reporting that it does not change (4,68,82,95,166,167, 170,189,249,356,357). However, rises (4,46,50,92,112,195,226, 259,267,271,272,273,274) and falls (4,7,60,111,156,196,220) in the blood sugar concentration have been found during exercise.

The glycogen stripping regimen in particular, appears to predispose to a fall in blood sugar during exercise (47,156,220), as confirmed by our results. Hultman and Nilsson (156) found levels as low as 1,8 mmoles/l at the end of 60 minutes of exercise following a carbohydrate-free diet; an hour later the blood sugar was still 1,9 mmoles/l. Our athletes' blood sugar concentrations were never less than 4,0 mmoles/l, but the nadir might have been missed, as TN had symptoms suggestive of an hypoglycaemic episode 1 - 2 hours after the glycogen-stripping exercise.

'Glycogen-loading' was found by Rennie and Johnson (175,273)

to increase the blood sugar concentration during exercise.

Athletic training was found by Devlin et al. (75), Karlsson et al. (183) and Winder et al. (356) not to affect the blood sugar concentration during exercise. But in all of Johnson's work (46,166,271,272), and in the investigation by Gyntelberg et al. (130) the athletes' blood sugar concentrations at the end of exercise had risen more than the non-athletes'. Our non-athlete's blood sugar also seemed to be less responsive to exercise than that of the athletes.

The blood sugar may rise or fall during exercise in patients with diabetes mellitus, depending largely on the resting pre-exercise concentration: it tends to fall if the initial concentration is less than about 18 mmoles/l, and to rise if it is higher than this (37,95,189,194,195,196,332).

Post-exercise ketosis seems to be suppressed if the blood sugar rises during exercise, and to be enhanced if it falls (46,166, 271,272,356), except in diabetes mellitus, where the opposite often occurs (37,194). But there is no correlation between the post-exercise levels of glucose and ketone bodies in the blood: in Johnson's work (166,271,272) the blood sugar levels of the athletes and non-athletes always converged during recovery, while the blood ketones diverged. There is also no correspondence between the post-exercise blood ketone body concentrations in our study and the blood sugar levels.

Effect of Exercise and Diet on Serum IRI

There have been a few reports of unchanged serum insulin levels during exercise (49,75,159,249), but the consensus in the literature is that the serum IRI level decreases during muscular activity (7,49,50,65,67,85,111,112,159,189,196,220,221,251, 259,267,273,295,331,357). The diminished insulin requirement of patients with diabetes mellitus on days when strenuous exercise is performed, is also well known (204,365).

Differences have been found between athletes and non-athletes in the extent to which their serum IRI levels fall during exercise,

but the differences are not consistent. Johnson et al. (177), Bloom et al. (46) and Gyntelberg et al. (130) found that non-athletes had lower levels than athletes, but Rennie and Johnson (272) and Rennie, Jennett and Johnson (271) found that it was the athletes who had the lower IRI concentrations during exercise. Devlin et al. (75) and Winder et al.(356) found that training had no effect on serum IRI levels during exercise. Whatever differences were found between athletes and non-athletes in all of these studies, the athletes usually had the higher blood sugar concentrations at the end of the exercise.

The serum IRI concentrations decreased in response to exercise in all the tests of our investigation. There was no correlation between the extent of the fall, however, and the type of diet or training which preceded the test, nor did the behaviour of the IRI concentration during and after exercise correlate with the degree or form of the post-exercise ketosis.

Oseid et al. (251), Böttger et al. (49,50) and Wahren et al. (331) found that the fall in serum IRI concentration during exercise was followed by a substantial rise during the first 5 - 20 minutes after exercise, before the level plateaued at about the pre-exercise concentration. This was missed in our investigation due to the infrequent blood sampling immediately after exercise.

Effect of Exercise and Diet on Serum HGH

Exercise is a stimulus for the release of growth hormone into the blood (55,75,85,249,251,259,271,316,318,319,320). There is often a lag of about 10 - 20 minutes before the serum HGH concentration starts to rise during exercise (55,137,203), but then it increases whether the exercise is continued or not. Thus, after 1 minute of intense exercise, the maximum HGH concentration occurs at about 30 minutes after the exercise (55). During more prolonged exertion the serum HGH concentration may reach a peak before the end of the exercise (55), and if the physical activity is continued for as long as 7 hours (158), several separate peaks in HGH concentration may be found

during the exercise period, suggesting that it is released in bursts, every 2 hours or so.

The serum HGH concentration usually falls fairly rapidly to normal at the end of exercise, but occasionally it may remain elevated for several hours (46,316). Adamson et al. (2) found that daytime exercise (before 14h00) significantly increased the midnight peak in HGH concentration, when the subjects were asleep.

The extent to which the serum HGH concentration rises in normal subjects, in response to exercise depends on the work load, diet and athletic training. There is an almost direct proportionality between the peak HGH concentration during exercise and the work load in all subjects (75,135,318,320), but, at a given work load, athletes usually have lower HGH concentrations than non-athletes (46,55,177,271,272,316,320). A low carbohydrate diet increases the HGH response to exercise (47), and a high carbohydrate diet decreases it (273). The ingestion of carbohydrate during exercise rapidly lowers the HGH concentration to normal (135,158), but the ingestion of fat or protein increases it (158).

The HGH response to exercise is greater in diabetes mellitus than in normal subjects (135,136). In rats the normal response to exercise would appear to be a fall in growth hormone concentration (87).

The HGH response to exercise has been found not to be influenced by the lactate, free fatty acid, or ketone body concentrations in the blood (137,172,180,318). HGH also does not appear to be necessary for the release of these metabolites into the blood, as patients with hypopituitarism mobilised these fuels in an exaggerated, rather than in a diminished manner during exercise (28,158,170,357).

Our study confirms that HGH is released during exercise, and that the concentration in the serum varies with the amount of carbohydrate that was in the diet on the days before the tests.

The HGH concentration during exercise was lower in the non-athlete than in the 2 long-distance runners, on a normal diet and after carbohydrate restriction. Devlin et al. (75) also found higher HGH concentrations in athletically fit subjects than in unfit subjects, but in view of the pulsatile nature of the HGH secretion during exercise, little significance can be attached to these exceptions. The serum growth hormone concentration followed a pattern quite different from the blood ketone body concentration during the post-exercise period.

Conclusion

Post-exercise ketosis was prevented by the addition of 60 - 90 g sucrose to the diet of the subject who had the highest blood ketone body concentrations after the exercise described in Chapter 5. Carbohydrate restriction intensified the post-exercise ketosis. A protein-fat diet caused 2 highly trained long distance runners to develop very high post-exercise blood ketone body concentrations (up to 3,88 mmoles/l). The serum free fatty acid, glucose, growth hormone and insulin concentrations followed patterns different from the blood ketone body concentrations during, and for 7½ hours after exercise, but they were also affected more, it seemed, by the composition of the pre-exercise diet than by training.

Post-exercise ketosis, previously attributed to a lack of training, could equally well be ascribed to the lower dietary intake of carbohydrates by sedentary subjects than by athletes.

CHAPTER 7

THE EFFECT OF ALANINE, GLUCOSE AND STARCH INGESTION ON THE KETOSIS OF EXERCISE AND STARVATION

Krebs (198), and others (107,211,212,230,337,339,347), have suggested that the metabolism of oxaloacetate in the liver determines whether acetyl-CoA is oxidised to carbon dioxide and water in the citrate cycle, or is converted to ketone bodies: acetoacetate and 3-hydroxybutyrate. They postulate that during starvation, diabetes mellitus and the recovery from exercise, oxaloacetate is preferentially decarboxylated to phosphoenolpyruvate as a gluconeogenic precursor, and is therefore unavailable for condensation with acetyl-CoA to form citrate. The resultant increased ketone body production is aggravated by the need to generate ATP for the gluconeogenic pathway by means of the partial oxidation of fatty acids (96,115,350), which theoretically gives rise to about 1,2 moles acetyl-CoA per mole glucose formed. Very little of this acetyl-CoA could presumably enter the citrate cycle, and therefore has to leave the liver as ketone bodies.

More recently, the emphasis has been on the hormonal control of ketone body production and utilization (11,185,186,187,233,235, 236,260,293,301,327,328). It is suggested that it is the plasma hormones which stimulate gluconeogenesis (a high plasma glucagon level on its own, or in combination with a low serum insulin), which also promote ketogenesis, through the release of free fatty acids from adipose tissue and channeling them into the oxidative pathways of the liver to form acetyl-CoA and ketone bodies instead of triglycerides. The gluconeogenic hormones are also believed to inhibit the peripheral utilization of ketone bodies (11).

On the other hand, the Oxford school (248,276,346) has suggested that the ketone bodies are important regulators of fuel homeostasis; high plasma concentrations having antilipolytic (23,31,42,99,172,173,238,300), antiproteolytic (257,304,305,324)

and antiglycolytic (22,23,238,324) actions which are important during intracellular shortages of carbohydrate. While it would be difficult to prove that vertebrates developed the capacity to become ketotic for this purpose, this hypothesis does suggest that the concentration of ketone bodies in the blood is carefully regulated, and that it would decrease if an increased rate of gluconeogenesis is associated with the replenishment of the body glycogen stores.

In this investigation we therefore compared the effects of ingesting the gluconeogenic precursor, alanine (90,91,93,311), with the effects of ingesting an equivalent amount of carbohydrate (glucose or starch) on the blood ketone body and gluconeogenic hormone concentrations of subjects rendered ketotic by prolonged exercise after a low carbohydrate diet (193), or by 65 hours of starvation. The results show that alanine has a significantly greater effect than carbohydrates in lowering the blood ketone body levels in both these conditions, indicating that ketogenesis is not the by-product of either the biochemistry or endocrinology of gluconeogenesis, but seems to be stimulated, possibly directly, by a low concentration of glycogen in the liver.

Method

The effect on the blood ketone body concentration of a 100 g oral dose of either alanine, glucose or starch was observed in 43 healthy men. Twenty of the subjects were highly trained long-distance runners who underwent 'glycogen stripping' as described in Chapter 6. Twelve of the subjects fasted for 65 hours, and 11 were studied on a normal day after a normal breakfast.

The subjects were all healthy, and were not taking medication at the time of the experiments. None had observed a change in body mass during the past year, nor had any of them had hormone therapy during that time. Their participation was voluntary, and they were fully informed of the intentions and possible risks of the tests.

The alanine (L-Alanine; Art. 1007 Merck Laboratories, Darmstadt), glucose (Dextrose B.P.; Lennon Laboratories, Port Elizabeth), or starch (Soluble Starch; Art. 1253 Merck Laboratories, Darmstadt) was always taken at 11h10. These substances were mixed with water and coffee (Instant Coffee and Chicory Extract; Pick 'n Pay No Name Brand) to improve their palatability. The subjects were encouraged to drink energy-free fluids, but were prohibited from eating food during the observation period. No one was allowed to smoke during the experiments.

A. Glycogen Stripping

Seventeen members of the University of Cape Town Running Club and 3 members of the Spartan Harriers Running Club were studied. They were all running at least 100 km per week, as part of their training programme.

For 48 hours before experimental days, the runners maintained their normal training but ate a carbohydrate-free diet consisting of meat, fish, eggs, green vegetables and unsweetened dairy products. On the experimental day they ate a standard breakfast (an egg, some cheese and a cup of milk) at 06h30 and then ran cross-country in groups of 2 - 3 for 2 hours from 07h00 till 09h00. The average distance covered was about 22 km. The mean outside air temperature at 08h00 was 13,7°C (range 6,7° - 24,0°C). It never rained while the subjects were out running. The rest of the day was spent indoors, doing sedentary office work.

At 11h10 the subjects were randomly divided, at first into 3 groups (a 'control', an 'alanine' and a 'glucose' group), but later, when the results became known, into 4 groups (to include a 'starch' group). Each person was then given 100 g alanine, glucose or starch to take by mouth, according to his group's designation. The members of the control group each drank a cup of unsweetened coffee without milk at 11h10.

One of the subjects took part in the investigation 3 times: first in a control, then in an alanine, and finally in a glucose experiment. Two other subjects took part in 2 of the tests, but the remainder performed only one experiment each. The ages, heights and masses of the subjects taking the different substances were as follows:

			Age (yrs)	Height (cm)	Mass (kg)
Control group	n = 6	mean	28	185	70
		range	20 - 48	176 - 191	64 - 78
Alanine group	n = 6	mean	24	177	69
		range	20 - 33	173 - 181	63 - 74
Glucose group	n = 6	mean	23	182	71
		range	18 - 29	171 - 193	57 - 85
Starch group	n = 6	mean	28	180	79
		range	21 - 37	176 - 188	64 - 77

Venous blood was taken from an arm vein at 07h00, and then at hourly intervals from 09h00 to 16h00, to determine the acetoacetate, D-3-hydroxybutyrate, free fatty acid, glucose, immunoreactive insulin (IRI), immunoreactive glucagon (IRG) and immunoreactive human growth hormone (HGH) concentrations.

B. Starvation

Twelve non-athletic students of physiology were studied after 65 hours of fasting, during which time they performed their normal daily activities, drank energy-free fluids, but ate no food and played no sports. On the third day of their fast they were arbitrarily divided into a control group and three test groups. At 11h10 the members of the first test group were each given 100 g glucose by mouth; the members of the second group, 100 g starch; and the members of the third group, 100 g alanine. The members of the control group drank a cup of unsweetened black coffee at 11h10.

The ages, heights and masses of the four groups of students were as follows:

			Age (yrs)	Height (cm)	Mass (kg)
Control group	n = 4	mean	19	178	66
		range	18 - 19	167 - 189	59 - 73
Glucose group	n = 3	mean	24	175	70
		range	19 - 27	167 - 184	62 - 85
Starch group	n = 3	mean	19	171	65
		range	19	166 - 173	62 - 68
Alanine group	n = 2	mean	20	183	71
		range	19 - 21	178 - 188	70 - 72

The subjects ate no food till 16h00, but performed their normal daily activities and drank energy-free fluids ad libitum.

Venous blood was taken from an arm vein at 11h00 (before the alanine, glucose or starch was ingested), and then at hourly intervals till 16h00 for the determination of the acetoacetate, D-3-hydroxybutyrate, free fatty acid, glucose, IRI, IRG and HGH concentrations.

C. Normal Diet

Eleven non-athletic students of physiology were studied on a normal working day, after they had eaten their usual breakfast. At 11h10 they were randomly assigned to two test groups and a control group, whose members were given 100 g of alanine or glucose, or a cup of unsweetened coffee, respectively. No food was eaten after that till 16h00, but they performed their usual daily activities and drank energy-free beverages ad libitum.

The ages, height and masses of the 3 groups of subjects were as follows:

			Age (yrs)	Height (cm)	Mass (kg)
Control group	n = 4	mean	18	181	78
		range	18 - 19	174 - 190	69 - 87
Glucose group	n = 4	mean	19	174	68
		range	18 - 20	169 - 183	62 - 72
Alanine group	n = 3	mean	19	188	83
		range	19 - 20	183 - 193	75 - 88

A fourth member of the alanine group vomited immediately after taking the alanine and did not take further part in the investigation.

Venous blood was taken by venepuncture at hourly intervals from 11h00 to 16h00, for the determination of the acetoacetate, D-3-hydroxybutyrate, glucose, IRI and IRG concentrations. The free fatty acid and HGH concentrations were not measured.

Results

Appendix 3. Tables 7-2 to 9-6.

General Observations

Prolonged exercise following 48 hours of the carbohydrate-free diet caused physical exhaustion and muscular weakness in all the athletes. This was particularly noticeable during uphill running. There were no symptoms suggestive of hypoglycaemia during or immediately after exercise, although one subject felt faint and sweaty about 3½ hours after the ingestion of glucose. His serum glucose concentration at 14h00, approximately 30 minutes before the onset of symptoms, was 2,0 mmoles/l. An hour later, when the faintness and sweating necessitated the early termination of the investigation, it was 3,7 mmoles/l. The subject felt normal again after eating a 50 g bar of chocolate and some sandwiches, and the episode was ascribed to a reactive hypoglycaemia.

No other subjects developed symptoms after the ingestion of glucose or starch. Alanine, on the other hand, caused abdominal discomfort on all the occasions that it was taken; in the runners this never became unacceptable, but in the subjects who fasted for 65 hours there was vomiting, in the one case within ½ hour, and in the other after 2 hours. As a result, it was decided not to give alanine to a third fasting subject, as was originally intended. This subject was instead assigned to the control group. A subject in the third set of

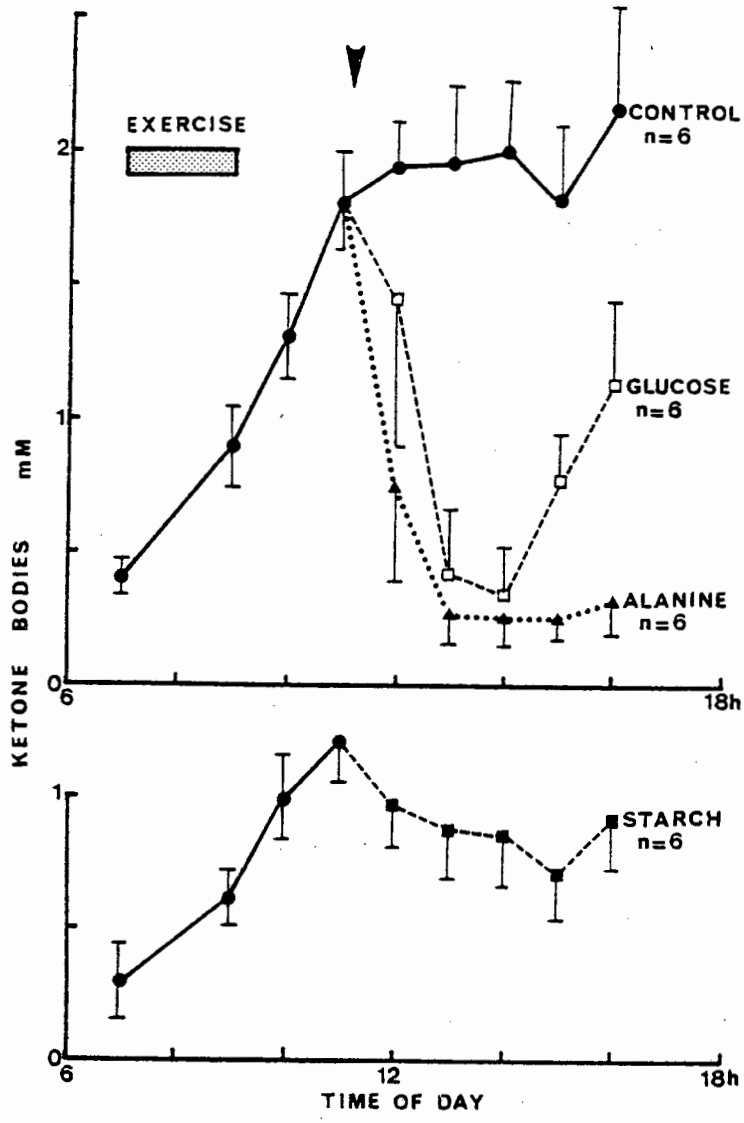

Fig. 26. The daily blood ketone body concentrations (mean ± SEM) of 18 trained athletes, who ran cross-country for 2 hours (07h00-09h00) following a "glycogen stripping" regimen, similar to that described in Fig. 21. At 11h10 (arrow) 6 of the subjects took 100 g alanine by mouth, 6 took 100 g glucose, and 6 took 100 g starch. The group who took the starch was not in the original research protocol, and since its mean 11h00 blood ketone body concentration is different ($P < 0,10$ - 2 tail t test) from the mean 11h00 value of the other groups, its results are displayed separately here and in Figs. 27-30.

experiments (the breakfasted group) also vomited immediately after ingesting alanine, and was excluded from the observations.

Ketone Bodies

When highly trained long distance runners exercised after taking a carbohydrate-free diet for 48 hours, the blood ketone body concentrations rose from a mean resting value of 0,41 (± SD 0,32) mmoles/l to 1,81 (± SD 0,81) mmoles/l at 2 hours after exercise. As in Chapter 6, the ketones then levelled off, dipped slightly and started to rise again 5 hours after exercise (Fig. 26).

The administration of 100 g glucose or alanine after exercise caused the mean blood ketone body concentration to fall to less than 0,5 mmoles/l in 2 hours. The fall was more prompt, less variable and longer lasting after alanine than after glucose ingestion. At 15h00 the blood ketone body concentration was rising again in the subjects who had ingested glucose, but not in those who had taken alanine. The difference in the mean blood ketone body concentrations of the two groups at 15h00 is statistically significant ($P < 0,01$).

As it was thought that these results might be due to a reactive hypoglycaemia occurring after glucose ingestion, it was decided to study the effect also of 100 g starch on post-exercise ketosis. Since this "starch group" was not in the original research protocol and because its mean 11h00 blood ketone body concentration was different (at the borderline of statistical significance: $P < 0,10$ - 2 tail t test) from the mean 11h00 value of the other groups, its results are displayed separately from the others in Figures 26 - 30. Starch ingestion caused the blood ketones to fall, but to a lesser extent than with alanine or with glucose ingestion. At 16h00 the mean ketone body concentration was rising again after starch ingestion at 11h10, as had been the case after glucose ingestion (Fig. 26).

Very wide variations were found in the blood ketone body concentrations of individuals who had fasted for 65 hours (0,59 - 5,18 mmoles/l), but the mean concentration (2,19 mmoles/l) was similar to the mean 11h00 blood ketone body concentration after exercise (1,81 mmoles/l). Glucose ingestion lowered the concentration to less than 0,1 mmoles/l in 3 hours, but at 16h00 the blood ketones were rising once more. The anti-ketogenic effect of starch ingestion was again much less pronounced than that of glucose (Fig. 31). The results of the administration of alanine are not depicted on Figure 31, because of the vomiting which occurred when this substance was taken after starvation, but they are included in Table 8-2 of Appendix 3. They show that the ketosis of starvation is reduced by alanine, but detailed comparisons with other studies are

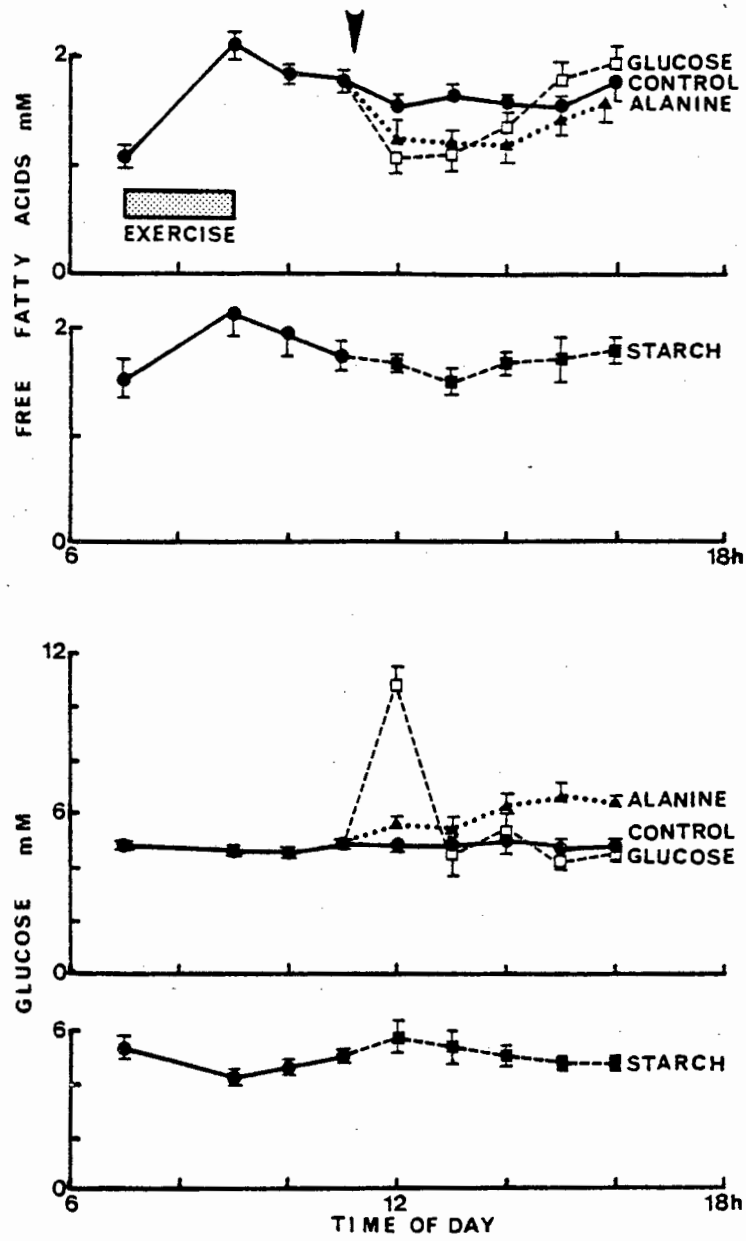

Fig. 27. The daily serum free fatty acid and glucose concentrations (mean ± SEM) of 18 trained athletes after running for 2hours, following "glycogen stripping". The experimental conditions and symbols are the same as in Fig. 26.

impossible, as it is not known how much alanine was absorbed, nor what the effects of vomiting are on the blood ketone body concentration.

The mean blood ketone body concentration at 11h00 after subjects had eaten their normal breakfast was 0,104 (± SD 0,108) mmoles/l; the level then gradually rose to 0,372 (± SD 0,307) mmoles/l at 16h00. Glucose and alanine lowered the blood ketone body concentration, and kept it below 0,07 mmoles/l until 15h00. At 16h00 the blood ketones started to rise in the glucose group, but not in the alanine group (Fig. 33); however, this difference

is not statistically significant ($P < 0,10$).

3-Hydroxybutyrate/Acetoacetate Ratio

Alanine, glucose and starch ingestion at 11h10 resulted in 3-hydroxybutyrate/acetoacetate ratios which were significantly lower at 12h00 than the corresponding control values ($P < 0,025$ to $<0,005$) in all 3 experiments (Figs. 30, 32 and 33).

Free Fatty Acids

The mean serum free fatty acid concentration rose from 1,07 ($\pm$ SD 0,43) mmoles/l to 2,11 ($\pm$ SD 0,53) mmoles/l during 2 hours of exercise in carbohydrate-starved runners, after which it fell to a plateau of about 1,6 mmoles/l in 3 hours. The ingestion of alanine or glucose lowered the post-exercise free fatty acid levels to approximately the same extent (the difference between the control level and the levels in the alanine and glucose groups at 13h00 is highly significant: $P < 0,005$), but the effect was short-lived. The effect of starch ingestion on the serum free fatty acid concentrations was minimal (Fig. 27).

The mean free fatty acid concentration after 65 hours of fasting was 2,20 ($\pm$ SD 0,31) mmoles/l. Glucose ingestion lowered the concentration to 1,00 ($\pm$ 0,05) mmoles/l in 2 hours ($P < 0,005$), but 3 hours later there was no statistical difference between the mean free fatty acid concentrations of the "control" and "glucose" groups. Starch ingestion, again, had only a minimal effect on the serum free fatty acids (Fig. 32).

Glucose

The serum glucose concentration was on average 3,9 ($\pm$ SD 0,8) mmoles/l in the control subjects who had fasted for 65 hours; 4,8 ($\pm$ SD 0,6) mmoles/l in the controls for the exercise experiments; and 5,8 ($\pm$ SD 0,4) mmoles/l in the non-fasting controls. In all 3 cases there was little variation in the serum glucose level during the observation period. Even the exercise in Experiment A did not significantly alter the serum glucose concentration.

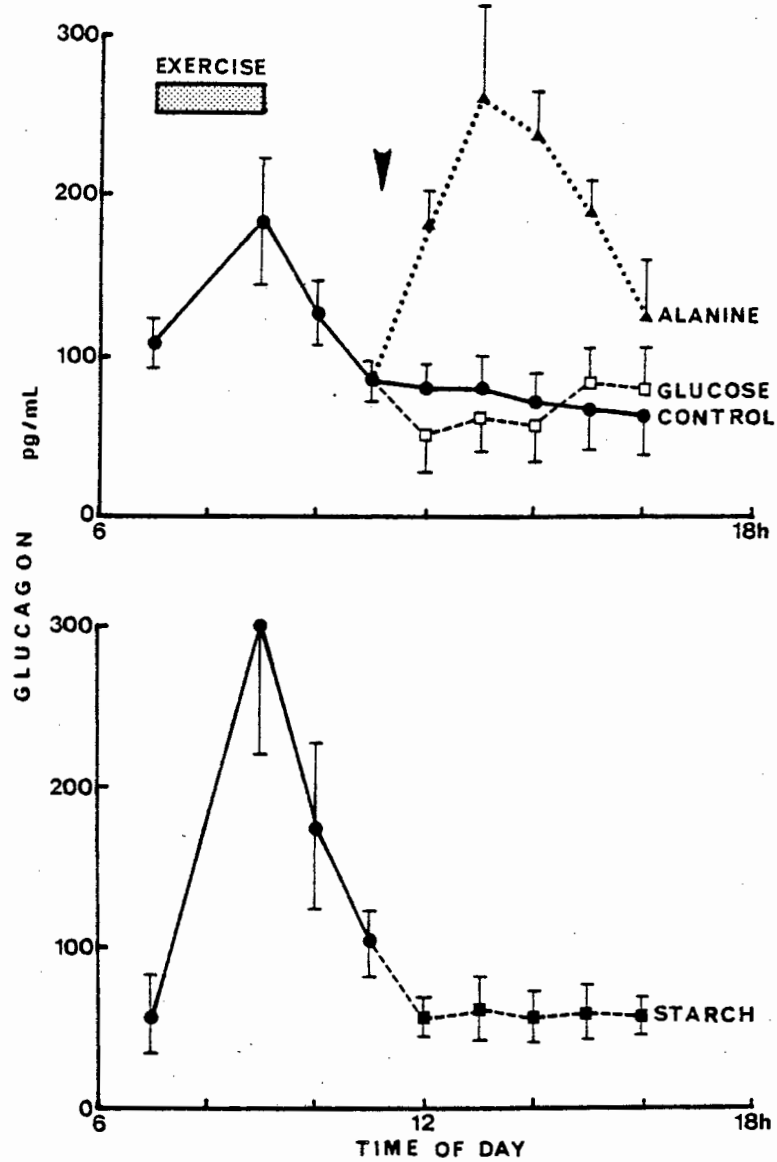

Fig. 28. The daily plasma immunoreactive glucagon concentrations (mean ± SEM) of 18 trained athletes after running for 2 hours, following "glycogen stripping". The experimental conditions and symbols are the same as in Fig. 26.

When 100 g glucose was given to normal breakfasted subjects at 11h10, the mean serum glucose level 1 hour later was only slightly higher (6,8 ± SD 1,9 mmoles/l) than that in the control group (6,0 ± SD 0,5 mmoles/l). This difference is not statistically significant (Fig. 33).

When glucose was given 2 hours after exercise, its concentration in the serum rose to 10,7 (± SD 1,7) mmoles/l in 1 hour, but was normal again in the next hour. A reactive hypoglycaemia (2,0 mmoles/l at 14h00) was seen in only one subject; in all the other subjects the serum glucose level remained above 3,9 mmoles/l (Fig. 27).

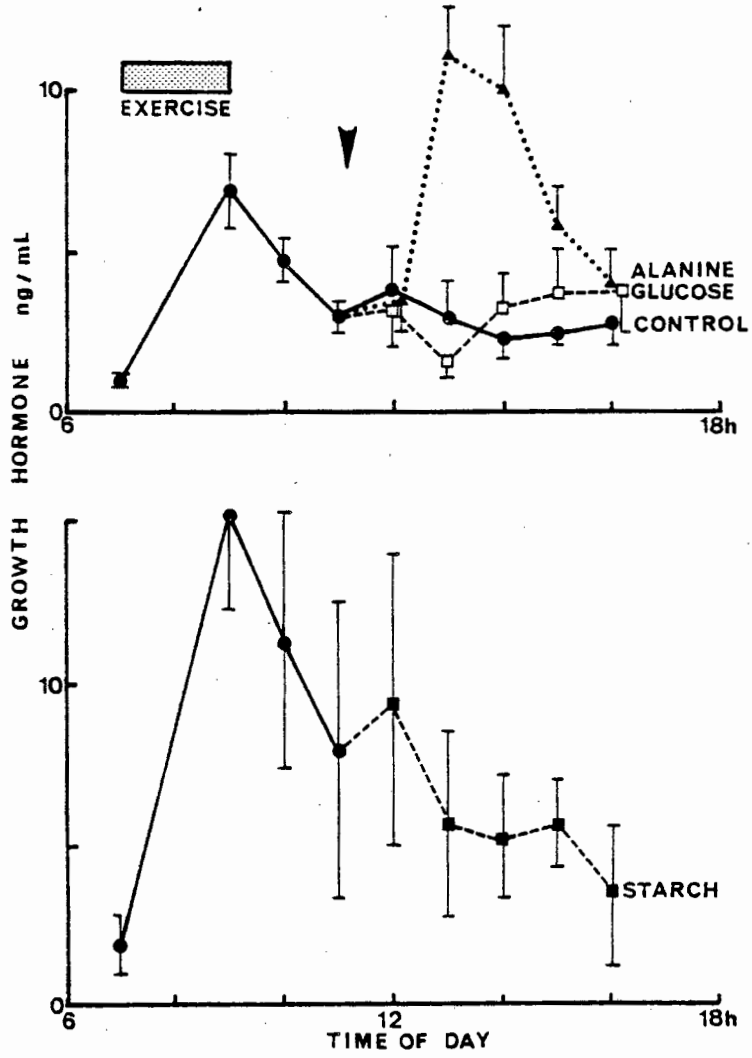

Fig. 29. The daily serum immunoreactive human growth hormone concentrations (mean ± SEM) of 18 trained athletes after running for 2 hours following "glycogen stripping". The experimental conditions and symbols are the same as in Fig. 26.

Starvation caused a marked glucose intolerance, with the serum glucose concentration rising to 10,5 (± SD 1,5) mmoles/l in 2 hours, and returning to normal only after 5 hours (Fig. 32).

The ingestion of alanine and starch caused mild, sustained increases in the serum glucose concentration, of usually not more than 3,0 mmoles/l, in all 3 experiments.

Immunoreactive Insulin (IRI)

The mean serum IRI concentration decreased significantly with exercise in Experiment A ($P < 0,0005$ - paired t test) (Fig. 30).

At 11h00 the mean serum IRI concentration was 9 (± SD 7) µU/ml

in the subjects who had exercised; 11 (± SD 3) μU/ml in those who had fasted; and 33 (± SD 34) μU/ml in the non-fasting subjects. Glucose administration caused steep increases in the serum IRI level in all experiments: the highest levels being found in the fasting subjects (108 ± SD 64 μU/ml at 13h00), followed by the breakfasted group (59 ± SD 35 μU/ml at 12h00), followed by the exercise group (49 ± SD 28 μU/ml at 13h00): but these differences are not statistically significant. The mean IRI concentration after glucose administration fell to below the control values at 15h00 in the post-exercise and breakfasted subjects, but not in the fasting subjects, whose IRI levels were still above control values at 16h00 ($P < 0,10$) (Figs. 30,32 and 33).

Alanine and starch had no discernible effect on the serum IRI concentration, except possibly in the non-fasting subjects, whose IRI concentrations fell more gradually after alanine ingestion than in the control group (Fig. 33).

Immunoreactive Glucagon (IRG)

Plasma IRG concentrations rose significantly during exercise in the carbohydrate-depleted athletes ($P < 0,0025$ - paired t test) (Fig. 28). The increase was greater in the 'starch' group than in the other groups of athletes, but the difference is not significant ($P < 0,10$ - 2 tailed t test). The IRG concentration started to fall immediately after exercise, to reach a level (84 ± SD 53 pg/ml) at 11h00 which was similar to that found in the breakfasted group (77 ± 43 pg/ml). The control values in both experiments then fell gradually to between 60 - 70 pg/ml at 16h00. (Figs. 28 and 33).

The fasting subjects' mean plasma IRG concentration was 214 (± SD 212) pg/ml at 11h00 (Fig. 32). Fasting control values fell during the observation period, but remained, at all times, significantly higher than the control values in the other experiments ($P < 0,05$).

Alanine proved to be a powerful IRG secretagogue after exercise

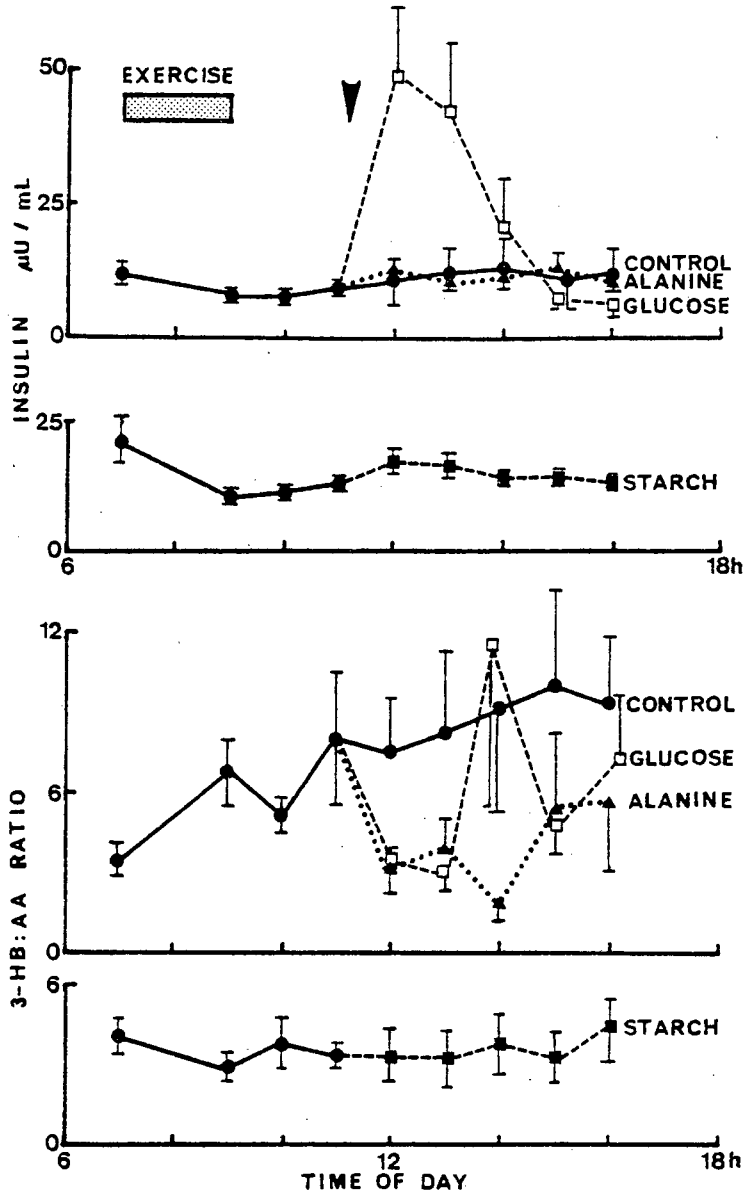

Fig. 30. The daily serum immunoreactive insulin concentrations and blood 3-hydroxybutyrate: acetoacetate (3-HB:AA) ratios (mean ± SEM) of 18 trained athletes after running for 2 hours following "glycogen stripping". The experimental conditions and symbols are the same as in Fig. 26.

(maximum mean IRG concentration was 258 ± SD 132 pg/ml), but produced only a mild elevation of the IRG levels in non-fasting subjects (maximum mean concentration was 118 ± SD 41 pg/ml). Although not all the alanine was absorbed by the 2 subjects who constituted the 'alanine group' in the fasting experiments - as they started vomiting after its ingestion - their mean IRG concentrations nevertheless rose to over 400 pg/ml at 12h00 and 13h00 (Table 8-8 in Appendix 3).

Glucose ingestion tended to lower the mean plasma IRG concentration, but this effect was not statistically significant, at any time, in any of the experiments ($P < 0,10$) (Figs. 28,32 and

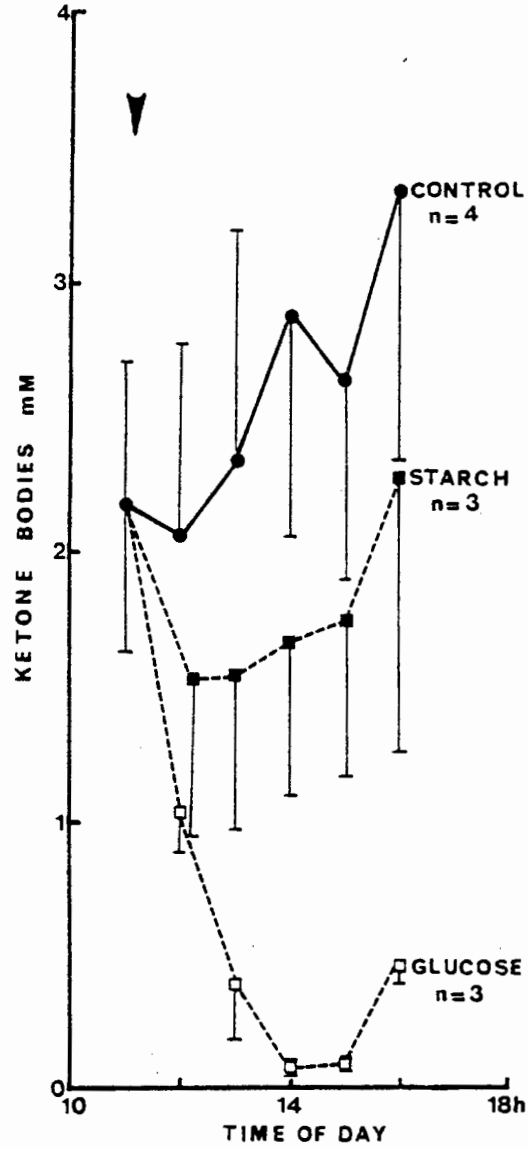

Fig. 31. The daily blood ketone body concentrations (mean ± SEM) of 10 non-athletic subjects who had fasted for 65 hours. No strenuous exercise was performed during the fast. At 11h10 (arrow) 3 of the subjects ingested 100 g glucose, and 3 of them ingested 100 g starch. Two other subjects (not depicted on the graph) took 100 g alanine by mouth, but vomited soon afterwards: their results are given in Table 8-2 of Appendix 3.

33). Starch produced inconsistent changes in the IRG concentration of the athletes and fasting subjects.

Human Growth Hormone (HGH)

The changes in the serum HGH concentration closely paralleled those of the plasma IRG concentration. The mean HGH concentration increased from 1,23 (± SD 1,36) to 9,10 (± SD 6,52) ng/ml with exercise in Experiment A ($P < 0,0005$; paired t test); the increase was significantly greater in the 'starch' group than in the other athletes, in spite of similar experimental

conditions up to this point ($P < 0,005$ - 2 tail t test) (Fig. 29).

Alanine ingestion caused a more than 3-fold increase in the serum HGH concentration after exercise, as well as after fasting, despite the vomiting which occurred in the latter situation (Table 8-9 in Appendix 3).

Glucose ingestion significantly lowered the HGH concentration in fasting subjects ($P < 0,05$ at 13h00, and $< 0,025$ at 15h00), but not in the post-exercise subjects. Starch produced equivocal changes in the serum HGH concentration in both the post-exercise and fasting subjects (Figs. 29 and 32).

Discussion

The results show that the ingestion of 100 g alanine was more effective than the ingestion of 100 g glucose at lowering the blood ketone body concentrations after exercise; with 100 g starch being only minimally effective in this regard. The differing antiketogenicity of these three substances did not correlate with any of the changes their ingestion produced in the plasma hormone concentrations. No qualitative differences were found to exist between post-exercise and starvational ketosis, supporting the suggestion first mooted by Courtice and Douglas (69) that the two conditions are probably expressions of a similar, if not identical, biochemical derangement in the body.

Alanine as a Substrate and Stimulus for Gluconeogenesis

Though there is considerable evidence that alanine is a primary substrate for gluconeogenesis in the body (21,86,88,91,93,281, 311), stimulating this process not only from its own carbon skeleton, but also from that of lactate, pyruvate, malate, fructose and glycerol (109,256), it has not been directly demonstrated *in vivo* that 100 g alanine ($\sim$1,1 moles) is quantitatively converted into 100 g glucose ($\sim$0,55 moles) as would be theoretically possible. Nevertheless, Ross *et al.* (278)

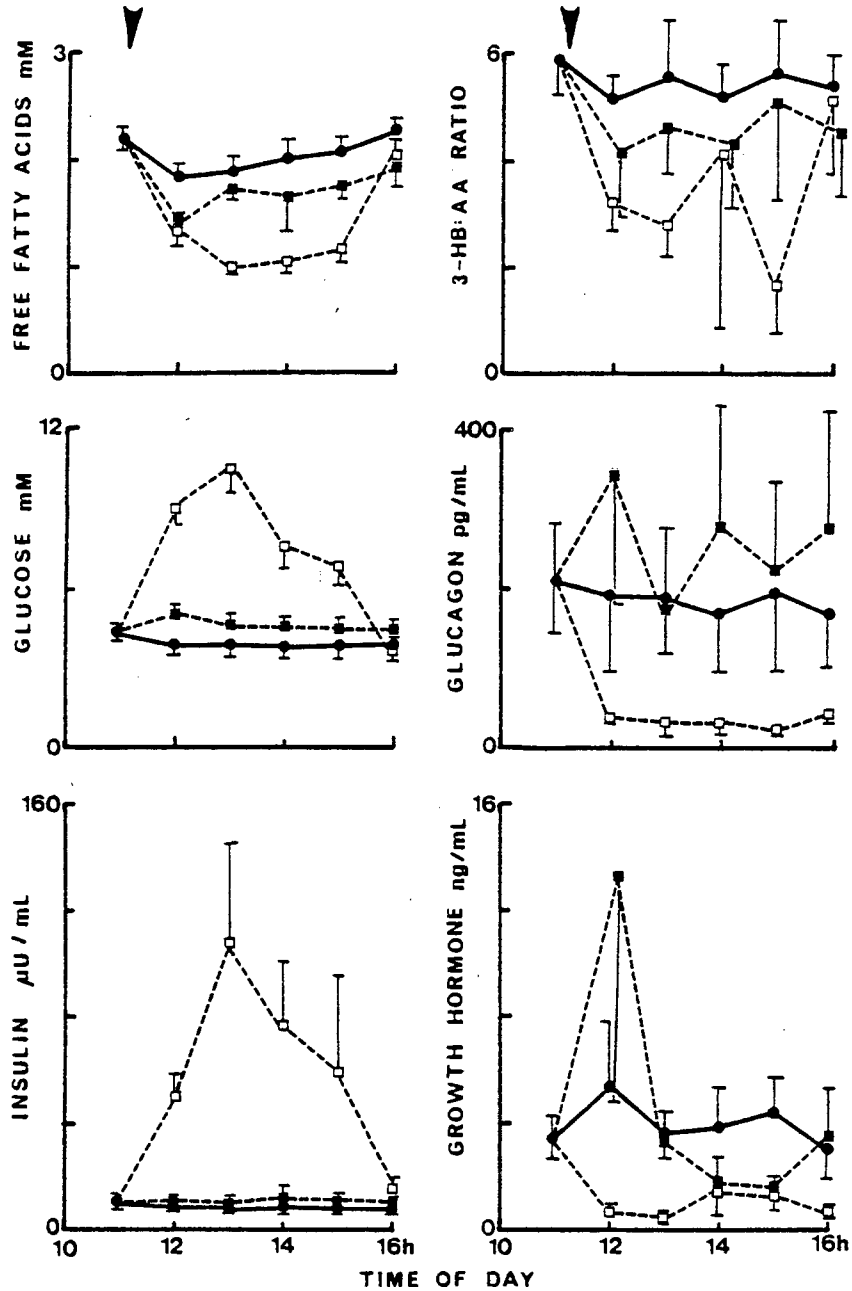

Fig. 32. The daily serum free fatty acid, glucose, insulin, growth hormone and plasma glucagon concentrations and the blood 3-hydroxybutyrate:acetoacetate (3-HB:AA) ratios (means ± SEM) of 10 non-athletic subjects who had fasted for 65 hours, without performing strenuous exercise. Experimental conditions and symbols are the same as in Fig. 31.

found that the rate of glucose formation from alanine in perfused rat livers was amongst the highest for any single amino acid, while Mallette et al. (224) and Sladek et al. (308) showed that this rate increased with increasing alanine concentrations without showing signs of saturation until an alanine concentration in the perfusate of about 20 - 30 times the normal plasma value was reached. The maximum gluconeogenic rate from most other amino acids occurred when the perfusate concentrations were about 3 times the normal levels (224).

Ozand et al. (256) showed that starved rats used alanine in preference to lactate for glucose and glycogen formation in the liver. It has also been found that alanine competitively inhibits hepatic pyruvate kinase (109,336), thereby blocking glycolysis and the recycling of phosphoenolpyruvate to pyruvate; thus increasing the efficiency of the new glucose formation.

Quantification of gluconeogenesis from ^{14}C-alanine requires a knowledge of the turnover rates of alanine and glucose, and of the precursor-product specific activity ratios during isotopic steady state conditions (200). The latter can be achieved only with constant infusion techniques, which, to our knowledge, have not been performed. Measurements simply of the incorporation of ^{14}C from uniformly labelled alanine into glucose and glycogen underestimates the rate of gluconeogenesis due to the randomisation of carbon atoms amongst the gluconeogenic intermediates and the pathway's various tributaries and branches (224). Thus oxaloacetate, for instance, forms a common pool for both new glucose formation and the tricarboxylic acid cycle, so that alanine carbons entering this pool would be used for both processes (199). Therefore, even if there was 100% net conversion of alanine into glucose, a proportion of the alanine carbons would be incorporated into CO_2, the amount of which depends on the relative rates of the gluconeogenic process and of the tricarboxylic acid cycle. Krebs et al. (199) estimated that in their experiments with kidney slices, the rates of the two processes were such that each molecule of oxaloacetate had a 2 - 3 times greater chance to enter the citrate cycle than to form phosphoenolpyruvate. This meant that even though almost as much glucose was formed as lactate (the gluconeogenic substrate used in these experiments) was consumed, about 57% of the lactate carbons were given off as CO_2. Sladek and Snarr (308), on the other hand, found that only 12% of alanine carbons were converted to CO_2, with the rest going to glucose, in liver slices from fed rats. Most in vivo studies have shown, however, that the incorporation of ^{14}C from labelled alanine into glucose is much smaller: between 12,5 and 28,0% in post-absorptive subjects (64,90,200), and up to 60% in 48 hour starved individuals (64,88,89).

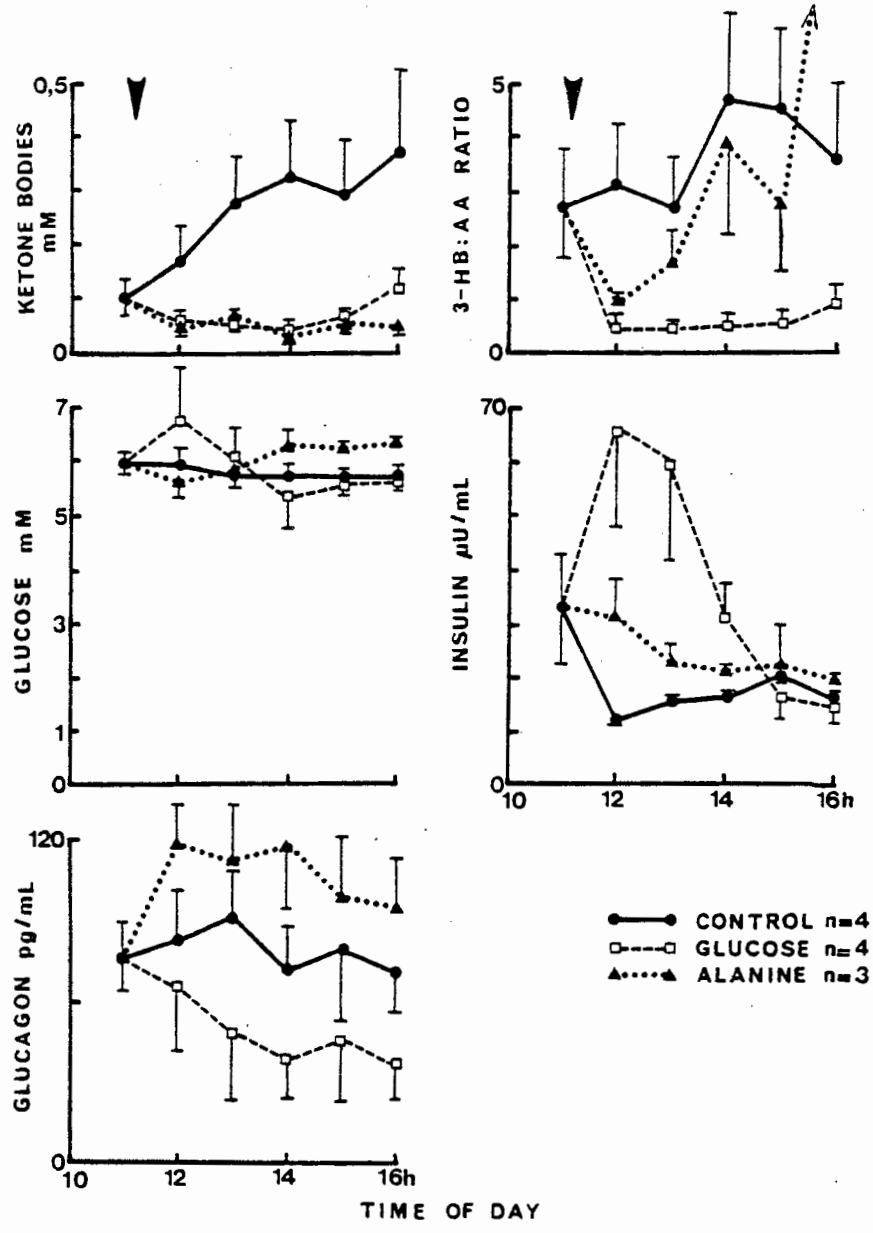

Fig. 33. The daily blood ketone body concentrations, 3-hydroxybutyrate:acetoacetate (3-HB:AA) ratios and serum glucose, insulin and glucagon concentrations (mean ± SEM) of 11 non-athletic subjects who had eaten a normal breakfast at about 07h30. No strenuous exercise was performed. At 11h10 (arrows) 4 of the subjects ingested 100 g glucose and 3 ingested 100 g alanine. A twelfth subject also took 100 g alanine by mouth at 11h10, but vomited immediately afterwards: he was excluded from the experiment. No food was eaten during the observation period.

The increase in the serum glucose concentration which occurred when our subjects ingested 100 g alanine (about 1,4 g/kg body mass) was in keeping with the findings of other investigators (20,21,56, 88,89,118,190,245,246,254,286,358), and has been ascribed by Saccà et al. (284) to an increased rate of hepatic glucose production when alanine is administered. Our results also confirm the

findings of Wise et al. (358,360) that the glycaemic response to alanine ingestion is proportional to the increase in IRG concentration which follows the ingestion of this amino acid (56,245,279,284,359). However, glucagon has been found in various studies not to affect the uptake of gluconeogenic precursors (e.g. alanine) by the liver, but rather to determine their fate within the hepatocyte. Thus Wahren et al. (333) found that somatostatin administration, which blocks IRG (and IRI) secretion, reduced the glucose output from the liver by 70% in post-absorptive and in 60 hour fasted subject, while leaving the combined uptake of endogenous lactate, pyruvate, glycerol and amino acids by the liver unchanged. Similarly, Chiasson et al. (62,63) found that an infusion of glucagon left the rate of alanine uptake by the liver unaltered, but doubled the rate of transfer of radioactive label from this amino acid to glucose.

It would thus appear that alanine's gluconeogenic activity depends to some extent on its ability to stimulate glucagon secretion (56,245,276,284,286,358,359), which, in turn, seems to vary with the subject's carbohydrate status. Thus we have found that alanine's stimulation of glucagon secretion is enhanced by starvation (Table 8-5, Appendix 3) (see also: 245, 358,359,360) and by exercise (Fig. 28). Other investigators have found that it is also enhanced by diabetes mellitus (358), but decreased by obesity (359,360). Alanine does not increase the plasma glucagon concentration in normal dogs rendered hyperglycaemic by means of a glucose infusion (245).

Antiketogenicity of Alanine

Alanine's prompt and maintained antiketogenicity in breakfasted and post-exercise subjects was more marked than that of glucose, in spite of alanine's modest effect on the serum glucose and IRI concentrations, and its stimulatory effects on HGH and IRG secretion.

In 1971 and 1973 Genuth (117,118) reported that a calorically insignificant daily dose of 50 g alanine reversed many of the

blood and urinary changes which had occurred in five massively obese subjects after two weeks of fasting: the ketonaemia, acidaemia and hyperuricaemia were reduced, while the blood sugar was raised; only the serum insulin level remained depressed. Potassium, phosphate, ammonium and 3-hydroxybutyrate excretion in the urine were decreased. Fasting natriuresis has also been found to be abolished by alanine ingestion (330). In 1974 Genuth and Castro (119) reported that the ketonaemia (2 mmoles/l) of untreated insulin-dependent diabetes mellitus was also reduced by alanine (0,5 g/kg body mass). This occurred in spite of rising free fatty acid concentrations, which, in normal and fasting subjects, as well as in maturity-onset diabetes mellitus, decreased when alanine was administered - as occurred in our study (Fig. 27, and Table 8-3 in Appendix 3).

Ozand et al. (254) found that the physiological ketosis of suckling rats was promptly reduced by the intraperitoneal injection of alanine (1 g/kg body mass). The administration of anti-insulin serum to 48 hour starved grown rats did not block the antiketogenic effect of alanine, nor did dichloro-acetate, which inhibits the peripheral utilization of ketone bodies (253). Alanine was found virtually to abolish the conversion of oleate to 3-hydroxybutyrate (256).

Kerr et al. (190) and Sann et al. (286) showed in 1978 that the blood 3-hydroxybutyrate concentration of malnourished and normal infants is lowered by alanine. Aynsley-Green et al. (20) found that 0,5 g alanine/kg body mass reduced the ketonaemia of a 9 year-old girl with glycogen synthetase deficiency.

Most of these authors have found that the antiketogenic effect of alanine was associated with a marked fall in the 3-hydroxy-butyrate / acetoacetate ratio in the blood, as also occurred in our experiments.

It is clear therefore that in all forms of ketosis, now including the post-exercise variety, alanine has a strong antiketogenic effect, which equals if it does not exceed that of glucose. It

is also clear from our results that glucose and alanine exert their antiketogenic effects in the context of widely divergent blood fuel-hormone pictures, and that alanine's antiketogenicity outlasts the hormonal responses to its ingestion.

Alanine's antiketogenic effects might be the result of its ability to supply oxaloacetate for the tricarboxylic acid cycle (198), which, once it is primed with this substance, will theoretically generate as much of it as it consumes, and therefore not make any further demands on the alanine supply. Any amount of lipid could then be oxidized to provide all the energy needed for the conversion of the remaining alanine to glucose, and more, without forming ketone bodies.

If this is indeed the mechanism whereby alanine suppresses ketogenesis, then it raises the question why a small - and it need theoretically be only a very small - portion of the hepatocyte's resources is not normally sequestered for this purpose during physiological ketoses, as oxaloacetate can be formed from many non-lipid sources other than alanine (198). One would therefore be forced to the conclusion that the liver synthesises ketone bodies as deliberately as it forms new glucose, triglycerides or serum albumin, and not merely as a by-product of gluconeogenesis.

It has recently been suggested that ketone body production in the liver is controlled at the level of entry of fatty acyl-CoA into the mitochondrion (11,231,235,236,276). This initial step in the β-oxidative pathway is catalyzed by carnitine acyltransferase, which is inhibited by high insulin and low glucagon concentrations in the blood, as well as by increased quantities of malonyl-CoA in the hepatocyte (11,232,235,236,276). Since the ingestion of alanine and glucose bring about opposite changes in the insulin and glucagon levels of the blood, but are likely to bring about similar changes in the intra-hepatic concentration of malonyl-CoA, the latter influence (on ketogenesis) would appear to dominate over the former.

On the other hand, if alanine is converted to malonyl-CoA in sufficient quantities to inhibit β-oxidation, where, then, does the fuel for gluconeogenesis come from? As has already been pointed out, alanine inhibits glycolysis (109,336), thereby cutting off that potential supply of ATP. This would mean that alanine itself (and possibly other amino acids) would have to be oxidized in the citrate cycle to provide the ATP for its own conversion to glucose. Or, alanine is not converted into malonyl-CoA in sufficient quantities to inhibit carnitine acyltransferase; and the block in ketone body production occurs at a later stage in the ketogenic pathway, possibly through a metabolite other than malonyl-CoA (see Chapter 8).

Another possibility is that alanine exerts its antiketogenic effects through some pharmacological action on, for instance, the autonomic nervous system or on cell membrane receptors to decrease ketone body synthesis and increase ketolysis. There is no evidence to support such a view, however.

We feel, however, that alanine's antiketogenicity is most likely related to its gluconeogenic properties: the ingestion of this amino acid supplying the body with sufficient gluconeogenic substrate to replenish vital carbohydrate stores, which, in their depleted state after exercise or starvation, were the stimulus for increased ketone body production. Ever since Hirschfeld (144) first demonstrated the fact in 1895, a reduced availability of carbohydrate has been recognised as being one of the main determinants of ketosis (20,107,198,228,232,233,242, 248,276,302,349). And today there is considerable evidence that raised blood ketone body levels are in fact signals of this state of affairs in the body, and that they bring about many of the metabolic changes which prolong life under these circumstances (248,276,346,348). The administration of alanine or glucose alleviates the carbohydrate shortage, and presumably operates a 'switch' (348) which stops further ketogenesis, as this has now become redundant.

Glucagon

Exercise after a low carbohydrate diet caused a marked increase in the plasma IRG concentration (Fig. 28). The level fell within two hours of the cessation of exercise to less than the pre-exercise level. A similar behaviour of the plasma IRG concentration during and after exercise has been found by many investigators (46,49,50,67,92,111,130,221,295,356). The IRG levels attained by our highly trained runners during exercise exceeded those of the untrained subjects in the studies of Bloom et al. (46), Gyntelberg et al. (130) and Winder et al. (356), suggesting that the differences found by these authors between athletes and non-athletes were probably due to the effects of training on their subjects' food intake. This is confirmed by Böttger et al. (50) and Ahlborgh et al. (8) who found that the hyperglucagonaemia of exercise could be suppressed by means of a glucose infusion before the exercise.

The metabolic role of glucagon during and after exercise was studied by Galbo et al. (112,113). When anti-glucagon serum was injected into rats after exhausting exercise, there were no changes in the liver and muscle glycogen stores, nor in the release of gluconeogenic substrates from the muscles; it also did not prevent the restoration to normal of the blood sugar level after exercise. But when antiglucagon serum was injected before exercise, the rate of hepatic glycogen depletion was halved compared with that in control animals.

Increased plasma IRG concentrations were found in our 65-hour starved subjects, confirming similar observations by other investigators (103,246,325,328). Since IRG concentrations are also raised in hypoglycaemia, diabetes mellitus, the neonatal period and after trauma and exsanguination (328), which are conditions frequently associated with ketosis, it has been suggested that glucagon has ketogenic activities. Support for this has been found in several in vitro studies which have shown that glucagon stimulates ketone body production in isolated liver preparations (52,139,239,314,329,351). However, the IRG levels in our fasting subjects were falling during the 5-hour observation period when ketone body concentrations were rising. This is in

keeping with the observations of Marliss et al. (225) that the plasma IRG levels are maximally raised only during the first three days of fasting, after which they fall to a plateau just above the post-absorptive level. Ketone body concentrations, on the other hand, usually continue to rise for many days before reaching a plateau during starvation (252).

It has been found that when glucagon is injected into patients with diabetes mellitus the blood ketone body levels increase (207,290,291), but when it is injected into normal fed subjects the ketone bodies usually decrease (207,292), though not always (289). IRG infusion during starvation has a variable effect on the blood ketones, and is liable to cause vomiting (225). The alanine-induced hyperglucagonaemias in our experiments were uniformly associated with reductions in the blood ketone body concentration. When the plasma IRG levels were suppressed by the ingestion of carbohydrates there were also reductions in the blood ketone body concentration. The plasma glucagon level by itself therefore does not play a direct role in the regulation of the blood ketone body concentration (110,186).

Insulin/Glucagon Ratio

A bi-hormonal system for the regulation of ketone body metabolism has recently been suggested by a number of workers (11,185,186, 187,233,235,260,293,327,328), who hold that it is not the absolute concentrations of either insulin or glucagon by themselves, but their molar ratio to each other, which determines the rate of hepatic ketone body production: a low insulin/glucagon ratio giving rise to ketosis, and a high ratio promoting antiketogenesis. The main evidence for this hypothesis has been the following:-

In 1975 McGarry et al. (232) showed that the hepatic ketogenic capacity of fed rats was markedly enhanced by anti-insulin serum and glucagon administration, when glucagon on its own was without effect on the plasma ketone body levels. Alberti et al. (10) showed in the same year that the development of

ketosis in insulin-dependent diabetics, deprived of their insulin, correlated more closely with the increase in the plasma glucagon level than with the increases in the cortisol, catecholamine or growth hormone levels. Gerich et al. (120) then showed that somatostatin administration, which blocks glucagon secretion, prevented the rise of the 3-hydroxybutyrate concentration in diabetics deprived of their insulin. These authors also showed that glucagon administration with insulin, in the presence of somatostatin, caused no change in the plasma 3-hydroxybutyrate concentration of insulin-deprived diabetics, whereas glucagon administration on its own increased the level markedly (121). Keller et al. (186) found in 1977 that the lowering of both the serum insulin and glucagon levels in 3-day starved dogs had no effect on hepatic ketogenesis, but when the insulin level was depressed on its own, ketone body production increased by 85%.

The insulin/glucagon ratio is thought to regulate ketone body metabolism by controlling lipolysis in the adipocyte, the relative activities of the re-esterification and fatty acid oxidation pathways in the liver, and the extra-hepatic utilization of ketone bodies (11,235).

Our results show however, that changes in this bi-hormonal system do not explain the antiketogenic effects of alanine, nor do they explain the ketosis of the post-exercise period. Glucose ingestion, certainly, produced a very marked increase in the insulin/glucagon ratio, and reduced the blood ketone body levels accordingly, in all our experiments. But alanine decreased the insulin/glucagon ratio through its strong stimulatory effect on glucagon secretion; yet it had an antiketogenic effect which, if anything, was greater than that of glucose. During the post-exercise period insulin levels remained more or less constant (Fig. 30), while the glucagon levels fell continuously (Fig. 28), giving rise therefore to a progressive increase in the insulin/glucagon ratio, which, instead of bringing about a fall in the blood ketone body concentration, as the bi-hormonal theory would have predicted,

was associated with a steadily increasing ketosis (Fig. 26).

Our results are, however, consistent with the hypothesis that a low insulin/glucagon ratio might stimulate gluconeogenesis (110, 225), but even this aspect of the bi-hormonal theory has been questioned by Sherwin et al. (306,307) and Lundbaek et al. (214).

Growth Hormone

The serum HGH concentration increased markedly when trained athletes performed prolonged exercise after eating a low carbohydrate diet for 2 days (Fig. 24), confirming the findings of the experiments described in Chapter 6. The serum HGH concentration also rose sharply when alanine was administered (see also: 119,225), but decreased when glucose was taken by mouth. The changes in the serum HGH level in all these experiments in fact closely paralleled those of the plasma IRG concentration. Avruskin et al. (19) found this to be due to a direct stimulatory effect of IRG on HGH secretion, causing the concentrations of these two hormones to vary in unison with one another in many experimental situations.

These results therefore exclude an insulin/glucagon/growth hormone combination from being the regulator of the blood ketone body concentration, as suggested by Alberti et al. (11), Gerich et al. (121) and Schade et al. (293,294). The possibility cannot be excluded, however, that the changes in blood ketone body concentration which occurred after exercise, and after glucose, starch and alanine ingestion were the result of pulsatile hormonal changes which were missed as a result of the hourly blood sampling. But it is difficult to imagine what form these hormonal pulses must have taken to account for the variety of ketone body responses seen here.

Other Hormones

The catecholamine and glucocorticoid concentrations in the plasma were not measured after exercise and alanine administration. Changes in the concentrations of these hormones might be important

in the regulation of the blood ketone body concentration, as the glucocorticoids, for instance, are the mainstay in the treatment of bovine ketosis (45,244), acting as stimulants for hepatic glycogen synthesis; though Schade et al. (293) list these hormones amongst the counter-regulatory hormones which stimulate ketogenesis.

Disposal of Glucose and Glucose Tolerance

When glucose is administered to subjects in the post-absorptive state, there is a change from a net output to a net uptake of glucose by the liver (89). The proportion of the glucose load which is taken up by the liver is relatively small, however: Radziuk et al. (270) estimated that only about 8% of the glucose absorbed from the gut was taken up during its first passage through the liver. Maehlum et al. (217) found a similar value for the total hepatic uptake in 1 hour (3 - 13%). But since hepatic gluconeogenesis (89,90,94,96) and glycogenolysis stop (270), the total splanchnic glucose output is increased by only 15 g in the 3 hours following a 100 g glucose load (94,97).

Most of this extra glucose output from the splanchnic area would appear to be taken up by adipose tissue, as Maehlum (219) found that the glycogen content of the muscles did not change in post-absorptive subjects after a 30 - 40 g glucose load, though Roch-Norlund et al. (277) found that much larger doses of glucose (500 - 600 g in 4 hours) could increase the muscle glycogen content by about 25%.

When 30 - 40 g glucose were given 15 minutes after exhausting exercise, the muscle glycogen content increased rapidly from 22 to 36 mmoles glucosyl units/kg wet mass in one hour (219). Maehlum estimated that this disposed of about half of the total glucose load (207), with 0,5 - 10% still being taken up by the liver (217). The rate of glycogen deposition in the exercised muscles was not appreciably different when 100 g glucose were given instead of 30 - 40 g (221), and was only slightly decreased when the glucose was given at 15 hours instead of at 15 minutes after exercise, despite the spontaneous increase (without food)

of the muscle glycogen content from 20 to 44 mmoles glucosyl units/kg wet mass during those 15 hours of recovery (220,221). The rate of muscle glycogen deposition was also not increased by injecting insulin into normal subjects (277), but it was decreased when diabetics were deprived of their insulin (222,277). The rate returned to normal when the diabetics were given their usual daily dose of insulin (218,222,277).

Yet, in spite of this avidity of the muscles for glucose, the glucose tolerance curve in our post-exercise subject was found to be disturbed (Fig. 27). This confirms the findings of Courtice et al. (69,70), and of Johnson et al. (167). Pruett and Oseid (266) also confirmed Courtice, Douglas and Priestley's (70) finding that this intolerance for glucose became manifest only 2 hours after exercise; the immediate post-exercise glucose tolerance curve showing an increased rate of removal of glucose from the blood (216). This improvement in glucose tolerance was found also in diabetic subjects (216), but only if they had insulin during the previous 17 hours (142). The reason is unclear, however. Oseid et al. (251), Böttger et al. (50) and Wahren et al. (331) found that there was a sudden, short-lived (10 - 20 minutes) increase in the serum insulin level immediately on cessation of exercise in human subjects and in dogs, but whether this could influence a 2-hour glucose tolerance test is not known, and it is certainly irrelevant to the findings in diabetics. However, Zinman et al. (364) found that the rate of endogenous glucose uptake and production remained elevated above pre-exercise levels for approximately 1 hour after cycling for 45 minutes, and fell to less than the pre-exercise levels only after the post-exercise peak in insulin concentration had passed; thus indicating that there are marked changes in carbohydrate metabolism associated with the changes in serum insulin level after exercise.

At 2 hours after exercise the serum insulin levels in our athletes were similar to their pre-exercise levels (Fig. 30), but lower than those of the breakfasted subjects (Fig. 33). Approximately the same serum insulin levels (50 - 60 U/ml) were attained by both groups of subjects after the ingestion of 100 g glucose, but

there was a marked difference in the concomitant blood sugar concentrations. This would suggest that there was a reduced tissue responsiveness to insulin after exercise, as also seemed to occur after the 65-hour fast (Fig. 32). Such tissue insensitivity to insulin has also been found in dogs on a low carbohydrate diet (34), in fasted rats (124) and in obese human subjects after 6 days without food (127); though most other investigators have found that it is an insensitivity of the pancreatic β-cells to glucose which gives rise to the glucose intolerance of fasting (12,56,72,73,100,122,323).

Whatever mechanism prevails during the post-exercise period, however, the rate of glucose uptake by the muscles is maximal (157), and cannot be increased by higher glucose or insulin levels in normal subjects (67,221,277). Liver glycogen synthesis probably also proceeds at its maximal rate. Thus, if there is glucose intolerance, it is almost certainly the result of a block to the entry of glucose into the adipocyte. Teleologically, this would serve the purpose of preventing the irretrievable loss of carbohydrate into fats, when the body's glycogen stores are critically low. Cahill (57,58) has proposed that such individual sensitivity adjustments to insulin in different tissues (muscle, liver, fat, etc.), under different physiological circumstances, are responsible for some of the metabolic changes seen with exercise, immobility, pregnancy, obesity, trauma, sepsis and ketoacidosis. He, in fact, proposes that the serum insulin concentration which is catabolically and anabolically neutral for any given tissue, changes with that tissue's state of activity, frequency of use and fuel content, as well as with the composition of the internal environment. Evidence for such insulin sensitivity adjustments has recently been presented by Koivisto *et al.* (196), and has been remarked upon by Conlee *et al.* (67) and Felig *et al.* (89).

Conclusions

Although 100 g starch is probably absorbed from the gut as 100 g glucose, its effect on the blood ketone body concentration resembled a diminutive version of what happened after glucose

administration more than it resembled a delayed glucose effect. Glucose's antiketogenic action therefore did not depend so much on its contribution of 100 g carbohydrate to the total body glycogen stores, as on the effects of a high serum glucose concentration on the distribution of those stores in the body (see Chapter 8).

Alanine's effect on the blood sugar level was closer to that of starch than of glucose, but its antiketogenic action was more like that of glucose than of starch. This would indicate that alanine was probably able to replenish critical (from a ketogenic point of view) carbohydrate stores without first being converted to blood sugar. If this was, in fact, the reason for alanine's antiketogenic action, it would follow that this carbohydrate store was situated in the liver, where most of alanine's conversion to carbohydrate presumably took place.

The importance of the glycogen content of the liver in the determination of ketone body metabolism has been recognised for a long time, even by those workers who have attributed hepatic ketogenesis to the actions of various hormones, or to incidental biochemical processes in the liver (20,198,210,228,232,242,263,276, 302,348,349,361). Within the scope of the parameters measured in our experiments (serum glucose, free fatty acid, IRI, HGH and plasma IRG concentrations) it is concluded that the hepatic glycogen content is probably the all-important factor which regulates ketone body production; with the blood fuel-hormone picture influencing the process only in so far as it reflects, or affects, the carbohydrate status of the liver.

CHAPTER 8

SYNOPSIS AND GENERAL DISCUSSION

In 1895 Hirschfeld (144) established that the amount of acetone excreted in the urine by non-diabetic subjects was almost exclusively dependent on the proportion of the diet which was carbohydrate, and in 1921 Shaffer (302) found that ketonuria occurred only if the non-nitrogen respiratory quotient (RQ) fell below 0,76. This, he calculated, represented the point at which equimolar quantities of fat (an arbitrary mixture of oleic acid, palmitic acid and stearic acid) and glucose were metabolised by the body; ketolysis predominated over ketogenesis when the molar ratio of glucose to fat in the combustion mixture was greater than 1, and vice versa when the ratio was less than 1. The conclusion from these and similar studies became summarised in the aphorism that 'fats burn in the flame of carbohydrate oxidation', and that when the flame is turned down low, the combustion of fats is incomplete and ketone bodies accumulate. Although this is no longer tenable as a description of the biochemistry of ketosis, it still serves to emphasise the importance of carbohydrate metabolism in the determination of whether a subject will become ketotic under a given set of circumstances or not.

The critical role of carbohydrate in the diet was reaffirmed in 1965 by Freund (107), who found that it was not the low total dietary energy intake which produced fasting ketosis, but the relative lack of carbohydrates: ketosis could be prevented if the fasting subjects ate a 4,2 MJ pure carbohydrate diet per day, but not if they consumed twice that amount of energy in the form of pure fats. In 1973 Genuth (117,118) showed that as little as only 0,85 MJ of energy per day in the form of pure glucose, was sufficient to reverse starvational ketosis.

It was our intention, in these studies, to find out whether post-exercise ketosis resulted from a lack of carbohydrates in the body after physical exertion. Previous experiments suggested

that this might be so: Courtice et al. (69,70) found that ketonuria developed only if the post-exercise non-protein RQ fell below 0,76. This was exactly the same RQ value which Shaffer (302) had found, 15 years earlier, to be the point below which nutritional and diabetic ketosis became manifest. Furthermore, post-exercise ketosis has been found to occur in subjects on high protein diets (229,265), high fat diets (14, 247), or on low carbohydrate diets (69,106,116), but never after they had been on a high carbohydrate diet.

In many investigations, post-exercise ketosis has, however, been found to occur in subjects on a normal mixed diet (see Appendix 1). It was chiefly the work of Johnson et al. (46,68,166,171,173,271,272) which suggested that these subjects developed ketosis after exercise as a result of a lack of athletic training. It was postulated (147,176,178) that the susceptibility of the non-athlete to post-exercise ketosis is due to the ketogenic blood fuel-hormone picture (high levels of free fatty acids, glucagon and growth hormone in the blood, and low serum insulin levels) which he develops during exercise; the exercising athlete's blood fuel-hormone concentrations showing much smaller deviations from the resting values than the exercising non-athlete's. However, these are changes which occur during exercise, whereas the ketosis is a characteristic primarily of the post-exercise period (69,85,164,165, 261), although this may simply be due to a lag-response.

Non-athletes also rely on glycolysis to a greater extent than athletes to provide the energy for muscular contraction (26, 141,146). This depletes the non-athlete's muscle and liver glycogen stores more rapidly than the athlete's: the latter using blood-borne free fatty acids as his chief source of energy for submaximal exercise (60,145,146,179,183,196). The low post-exercise liver and muscle glycogen content could presumably also stimulate ketone body production, but this has not been confirmed by direct measurements of the glycogen stores during various grades of post-exercise ketosis.

Our first experiments were designed to obtain supportive

evidence for this latter postulate. Athletes (long distance runners and competitive swimmers) and non-athletes were asked to swim underwater for as far as they could go at 07h30 on the first of 2 test days. At 07h30 on the next experimental day, a week later, they swam the identical distance on the surface of the water without breath-holding. The blood ketone body and lactate concentrations before, and for 9 hours after the swim were compared with control day values when the subjects did not exercise at 07h30. No food was eaten on the test or control days, but the subjects were encouraged to drink energy-free beverages to remain in fluid balance during the period of observation. The pre-exercise diet was not controlled.

It was hoped that during the underwater swim, athletes and non-athletes would use up similar quantities of muscle glycogen, as the diving reflex (13,298,362), elicited by the breath-holding underwater, was expected to produce intense peripheral vaso-constriction in all subjects (13,83), thus depriving the muscles of athletes and non-athletes alike, of nearly all blood-borne fuels. Swimming without breath-holding was expected not to elicit the diving reflex (16,148,297), thus permitting the athletes to burn free fatty acids in preference to carbohydrates during exercise, to conserve their body glycogen stores. The non-athletes were expected to continue to rely mainly on glycolysis for their energy production during the swim on the surface of the water. The results were equivocal, however, as none of the subjects developed post-exercise ketosis after either form of the exercise (Chapter 3).

Similar results were obtained when athletes and non-athletes worked at, or near, their maximum aerobic capacity for 15 minutes, and also when they performed exercise at heart rates of about 125 beats/minute for up to 90 minutes (Chapter 4).

These results were in marked contrast with Johnson's studies in Glasgow, in which untrained subjects showed marked degrees of ketonaemia, though with wide individual variation, after exercise lasting from 20 to 90 minutes (46,61,68,166,167,168,169,170, 171,173,271,272,273). This difference between our results

and Johnson's is unlikely to be due to differences in physical fitness between the national groups, since the fitness indices (as estimated by the Harvard Step Test) of young men in Cape Town and in Glasgow is approximately the same (237), and the unfitness of the untrained South Africans is shown by their high exercising heart rates (Fig. 9) and blood lactate concentrations (Fig. 11). Post-exercise ketonaemia occurred in some of the older South African subjects, but the response was very variable (Fig. 19). These older subjects were not less fit than the younger sedentary subjects, as their heart rates were the same for the equivalent work load (Chapter 5).

As the subject who had developed the highest post-exercise blood ketone body concentrations after exercise ate almost no refined sugars in his normal diet (e.g. no sugar in his tea or coffee, no candies and no pudding etc.) it was decided to find out what effect adding sugar to his tea and coffee would have on the ketosis he developed after exercise. There were 6 test days, each a week apart, on which this 37-year old subject cycled at 100 W for 2 hours (07h00 to 09h00). Prior to the first test he ate his normal diet, which included about 250 g carbohydrate per day, mainly as starch in bread, potatoes and rice. For 2 days before the second and third tests he added a total of about 60 - 90 g sugar (sucrose) to his diet per day, as sweetener for his tea and coffee. The fourth and fifth tests were each preceded by 2 days of carbohydrate restriction (total carbohydrate intake of about 80 g/day). The sixth test was carried out after he had returned to his usual diet for a week. The results showed that the addition of the 60 - 90 g sucrose to the pre-exercise diet abolished the post-exercise ketonaemia which this subject developed on his normal diet, whereas a low carbohydrate diet intensified it (Chapter 6).

These results were compared with what happened when 2 highly trained marathon runners exercised for 2 hours (07h00 to 09h00) after training on a low carbohydrate diet for 48 hours (the so-called 'glycogen stripping' regimen (5)). This produced blood ketone body levels which reached 3,88 mmoles/l at 16h30 in one of the subjects, the highest level of ketosis yet to have been

reported after exercise (Fig. 21). It was clear therefore that neither the intensity nor the duration of exercise, nor the level of training were as important as the diet (and presumably the body's glycogen stores) in determining the blood ketone body levels after exercise (Chapter 6).

That our untrained younger subjects did not develop post-exercise ketosis, when Johnson's subjects did, can therefore probably be ascribed to differences in dietary habits between the subjects in Glasgow and Cape Town. That such differences might have existed, is further corroborated by the finding that the resting blood lactate levels were significantly higher in our non-athletes than in our athletes (Figs. 3 and 11) whereas in Johnson's studies it was always the other way around (166, 271,272). As discussed in Chapter 2, this is most likely to be due to a proportionally greater amount of carbohydrates in the diet of our non-athletes than in that of the athletes; the converse presumably being true for the athletes and non-athletes in Glasgow, thus predisposing the sedentary Glaswegians to ketosis when they performed unfamiliar exercise. Our athletes did not become ketotic after exercise, probably because of the large absolute amounts of carbohydrate in their diet (the 2 marathon runners described in Chapter 6 were estimated to consume, on average, twice as much carbohydrate per day as the non-athletic subject who developed the highest post-exercise blood ketone body levels in Chapter 5).

A further finding in this investigation (Chapter 6) was that when post-exercise ketonaemia did occur, the variations in the blood ketone body concentrations were qualitatively different from the variations in any of the other parameters studied: thus, although the serum free fatty acid levels rose during exercise, reaching peak values on cessation of the work, or shortly thereafter, they had returned to their pre-exercise levels when the ketone bodies were at their highest. Serum glucose concentrations were also unrelated to the behaviour of the ketone bodies. The post-exercise ketone body levels also did not reflect the changes in any of the blood hormone concentrations measured here. Further confirmation that the

blood ketone body concentrations can rise or fall independently of the serum free fatty acid, glucose, insulin, growth hormone and plasma glucagon concentrations was obtained when 100 g glucose, alanine or starch were given by mouth to non-diabetic subjects rendered ketotic through exercise after a low carbohydrate diet, or through 65 hours of starvation (Chapter 7). Glucose administration caused an increase in the insulin/glucagon ratio, whereas alanine depressed it, yet both had similar antiketogenic effects, reducing the blood ketone body concentration from about 2 mmoles/l to about 0,2 mmoles/l in 2 - 3 hours. The changes in serum growth hormone level in all these experiments closely paralleled those of the plasma glucagon concentration, thus also excluding an insulin/glucagon/growth hormone combination from being the regulator of the blood ketone body concentration, as suggested by Gerich et al. (121) and Schade et al. (293,294).

It was also found that the changes in the serum free fatty acid, glucose, insulin, glucagon and growth hormone concentrations during exercise varied, as did the blood ketone bodies, with the previous days' carbohydrate intake. This indicates that many of the hormonal and biochemical differences previously observed between exercising athletes and non-athletes (130, 271,272,356) were probably due more to the athletes' higher food intake, than to their altered cellular metabolism. Thus, when the non-athletic subject in Chapter 6 ate a high carbohydrate diet for 2 days, he showed all the minimal blood fuel-hormone concentration changes with exercise, which are considered to be the hallmark of the highly trained athlete; while the marathon runners developed the low serum insulin levels and high free fatty acid, glucagon and growth hormone concentrations normally associated with the untrained state, when they exercised after eating a low carbohydrate diet for 2 days. (Chapters 6 and 7).

This does not mean that there are no qualitative differences between the fuel preferences and metabolic responses of trained and untrained muscles. Henriksson (140), for instance, found that when subjects performed 2-legged exercise after training

only 1 leg for 2 months, the trained limb consumed free fatty acids, but the untrained limb did not, in spite of receiving the same arterial blood. The trained limb also had a lower respiratory quotient (0,91 compared with 0,94) and produced less lactate during the exercise than the untrained limb. It was also noted that the trained limb performed the greater share of the total work done on the bicycle ergometer, consuming 11% more oxygen and producing 30% more force on the pedals than the untrained leg. The differences in fuel preference between the 2 legs would presumably have been accentuated if both limbs had performed the same amount of work (285). What our work has shown, however, is that there is another aspect to athletic training which involves the eating of more food per day (74,227) and storing particularly the carbohydrates (321) in such a way that only minimal hormonal changes will release it during exercise. When unusually strenuous training is undertaken (295), or when the diet has been deficient in carbohydrates (47), the athlete resorts to the same mechanisms used by the non-athlete to maintain the blood sugar level during exercise: namely, breaking down structural body elements to release substrates for gluconeogenesis. This is probably regulated by the blood insulin, glucagon, growth hormone, catecholamine and glucocorticoid levels (47). If the non-athlete increases his carbohydrate intake, he too can increase his liver and muscle glycogen stores (39,154,155,156) and avoid the diabetic-like blood hormone picture which he normally develops during the performance of unfamiliar exercise.

The question we tried to answer with our final experiments was whether post-exercise ketosis was the result of the lowered liver and muscle glycogen content after exercise, or of the gluconeogenesis (95,197) required to maintain the blood sugar level, when inadequate carbohydrate had been stored for the exercise undertaken.

We therefore gave 100 g alanine, glucose or starch to athletes who had become ketotic from 2 hours of running after training on a low carbohydrate diet. The alanine, which has been shown to be one of the prime substrates and stimulators of gluconeogenesis (21,86,88,91,93,281,311), was expected to be used to

replenish the depleted glycogen stores through new glucose formation. Its ingestion would therefore increase the ketosis if it was this process which gave rise to ketogenesis (115,198,211, 212,230,337,339,347), but decrease it if it was the low glycogen stores which stimulated ketone body production (198,210,232,233, 242,263,276,348,349,361). The equivalent amount of carbohydrate was also given directly as glucose or starch, for comparison (Chapter 7).

The results showed that the ingestion of 100 g alanine was more effective than the ingestion of 100 g glucose at lowering the blood ketone body concentration after exercise; with starch being only minimally effective in this regard. This indicated not only that ketogenesis and gluconeogenesis are completely dissociated processes in the liver, but also the degree of ketonaemia is determined more by the distribution of carbohydrate in the body than by its total amount. This last conclusion is based on the assumption that glucose and starch both enter the body as glucose, but at different rates. Such differences in the rate of absorption of glucose from the gut are likely to affect not only the blood glucose/tissue glycogen ratio of the body, but possibly also the disposal of glucose from the blood into the various tissues; it being quite possible that a high portal vein blood glucose concentration leads to a greater proportional extraction of glucose by the liver than does a low portal vein blood glucose level (94). Since it is unlikely that the ingestion of 100 g alanine contributed as much carbohydrate, and as quickly, to the body as did the ingestion of 100 g glucose, the superior antiketogenic effects of alanine must therefore also be ascribed to the replenishment of a critical glycogen store. As most new glucose formation occurs in the liver, it is likely that its glycogen store will be replenished first when alanine is administered; and, this being so, that it is the liver glycogen content which determines the rate of ketone body production.

The role of the glycogen content of the liver in ketogenesis was debated during the General Discussion at the 1978 International Symposium on "The Biochemical and Clinical Aspects of

Ketone Body Metabolism" in Reinhausen: Williamson (348) mentioned that children with Type I glycogenosis, who are unable to break down liver glycogen due to an absence of glucose-6-phosphatase, do not become ketonaemic during starvation in spite of developing severe hypoglycaemia (1,9 mmoles/l), hypoinsulinaemia (3,5 to 6,0 µU/ml) and massive hyperglucagonaemia (5000 pg/ml). On the other hand, children with a glycogen synthetase deficiency (20) do not become ketotic while they are eating regularly during the day, but they do develop blood ketone body concentrations of 8 mmoles/l and more overnight, when their blood sugar levels drop to 1,0 - 1,5 mmoles/l. McGarry (234) pointed out however that he had found no hint of an increased rate of ketogenesis from oleate during experiments in which the glycogen level of perfused livers was reduced with glucagon.

These contrasting data could, however, be reconciled by supposing that the intrahepatic 'switch', proposed by Williamson (348), for the regulation of ketone body synthesis is operated not by the liver glycogen content itself, but by the concentration of an intermediary in hepatic glycogen metabolism, such as glucose-6-phosphate or glucose-1-phosphate: high levels 'switching' ketogenesis off, low levels 'switching' it on. The concentrations of these intermediaries are likely to be permanently high in children with Type I glycogenosis, preventing ketosis even when the blood sugar level falls to less than 2 mmoles/l during starvation. In children with a glycogen synthetase deficiency they are likely to be high after meals, but very low during an overnight fast, producing the marked circadian variations in blood ketone body concentrations seen in these subjects. The levels of glucose-1-phosphate and glucose-6-phosphate in McGarry's perfused livers may not have changed while there was active glycogenolysis during his 1 hour experiments; and this may also be the reason why the exercise period itself (when there is active hepatic glycogenolysis) is frequently associated with a relatively constant, or even falling, blood ketone body concentration (24,30,46,60,79,80,114,134,150,164,247,301), whereas the post-exercise period (when hepatic glycogenolysis

presumably stops) is almost invariably associated with an increase in the blood ketone body concentration (see Chapter 1). Direct supportive evidence for this hypothesis has yet to be found, however.

On the other hand, there is evidence that another metabolic intermediary, malonyl-CoA, provides a link between ketogenesis and the hepatic carbohydrate status (11,231,235,236,276). This substance is made chiefly from carbohydrate-derived acetyl-CoA during the initial steps of fatty acid synthesis in the liver. High concentrations of malonyl-CoA, which, under physiological circumstances would result from an abundance of carbohydrate in the hepatocyte, have been found to block the transfer of fatty acyl-CoA from the cytosol into the mitochondrion, thus preventing β-oxidation and the formation of fatty acid-derived acetyl-CoA and ketone bodies. This block is brought about by the inhibition of carnitine acyl-transferase, which would appear to be sensitive also to the insulin and glucagon concentrations of the plasma (11,231,232,235,236).

The evidence that such a reciprocal relationship exists between the lipogenic and β-oxidative pathways in the hepatocyte is well founded (36,236), but that it explains the sensitivity of the ketogenic process to the liver glycogen content seems doubtful in our view: the link between the glycogen content of the liver and the concentration of malonyl-CoA appears excessively indirect, as does the link between the activity of carnitine acyltransferase and ketone body production. This hypothesis, as it stands, assigns, in fact, only a minor role to the hepatic glycogen content in the regulation of ketone body synthesis, whereas our work (and, we believe, the burden of accumulated experience in ketosis since 1895) suggests that ketogenesis is primarily determined by the liver glycogen content, with the blood hormone and free fatty acid concentrations having only an indirect, secondary or coöperative influence.

Infusion of 3-hydroxybutyrate or acetoacetate into post-absorptive, fasting or diabetic subjects, or into animals, results in a reduction of the serum glucose (23,98,172,173,215,238,300,

304,305,343), free fatty acid (23,31,42,99,172,173,215,238,300), glycerol (99,173,300) and alanine (304,305) concentrations. These changes have been shown to be associated with widespread metabolic alterations which conserve fuel and spare protein (248,276,304,305,310): hyperketonaemia reduces the output of glucose from the liver (238), and inhibits its utilization (98,215,238) in the periphery by reducing glycolysis and glucose oxidation (282), but not by affecting glycogen deposition (223).

High concentrations of ketone bodies in the blood also reduce the rate of proteolysis in muscle (257,304), and the rate of lipolysis in adipose tissue (42). These actions may be enhanced by the stimulatory effects of ketone bodies on insulin secretion (31,42,215), though in most naturally occurring ketoses the insulin level is low.

These actions of 3-hydroxybutyrate and acetoacetate have recently been extensively reviewed by Robinson and Williamson (276), whose conclusions are that ketone bodies act as signals of a lack of carbohydrate in the body, and help to regulate whole-body metabolism under these circumstances. Since exercise depletes the body of carbohydrate, it is likely to cause ketosis, the intensity and duration of which will depend on the severity of the carbohydrate shortage, and on whether the shortage can be relieved by a redistribution of fuel within the body, or requires the intake of new fuel by mouth. The monophasic post-exercise ketonaemia seen in Chapter 5 would, in this view, be compatible with a small, predominantly hepatic, glycogen shortage, which was correctable within hours by gluconeogenesis. The protracted, biphasic response seen after dietary carbohydrate restriction in the subjects of Chapters 6 and 7 would indicate a more general depletion of the glycogen stores which could not be corrected by a redistribution of depot fuels alone.

APPENDIX 1

SUMMARY OF LITERATURE ON POST-EXERCISE KETOSIS IN NON-DIABETIC SUBJECTS

Explanation of Abbreviations used in the table

Author column: The name given is that of the first author of the article. When a series of articles have appeared from a single centre(e.g. Southern General Hospital, Glasgow) the re-appearing author's name (e.g. R.H. Johnson) is quoted for the whole series.

Subjects column: The subjects are human unless stated otherwise. "Normal" refers to adults who were randomly chosen from a healthy population. "Trained" or "training" refers to athletic endurance training - usually long-distance running.

Diet column: "Normal" refers to the subjects' usual diet. In most of the investigations the subjects performed the exercise in the "post-absorptive" state - after an overnight fast.

Ketone bodies column: The figures given are mean values only; extracted from the reports as accurately as the presentation of the data permits.

"$\dot{V}_{O_2}$ max.": Maximum oxygen consumption.

Author and Date	Ref.	Subjects	Diet	Exercise	Urinary Ketone Bodies			Time after exercise (minutes)
					Before exercise	On cessation of exercise	During recovery	
Hirschfeld 1895	144	2 labourers	low carbo-hydrate	manual labour for 4 hours (at heart rate 120 beats/minute)	no increase in acetonuria in post-exercise urines			
Forssner 1909	106	1 normal	low carbo-hydrate	4 km walk at 6,5 km/h	increased acetonuria on exercise days			
Ryffel 1909	283	competitors in Black-heath Har-riers race	normal	24 hour track walking race		ketonuria in all subjects		
Barach 1910	29	19 trained subjects	normal	42 km marathon race	no ketonuria	ketonuria in 18 of the 19 competitors		
Preti 1911	265	1 normal	high protein	running up and down stairs till exhausted	increased acetonuria on exercise days			
Scott-Wilson 1911	299	1 normal	normal	normal daily activities	urinary excretion of acetone raised on some, but not all, days on which the subject "exerted" himself			
Hill 1924	143	1 marathon runner	normal	24 km run at 13 km/h		ketonuria		
Liljestrand 1925	208	3 normal	normal	2-3 minutes stairs running	no ketonuria	no ketonuria	no ketonuria	60
McClellan 1928	229	3 normal	pure meat	5-8 km walk	"high rates of ketone body excretion a few hours later"			
Courtice 1936-1939	69 70	1 normal	slight carbo-hydrate restric-tion	16 km walk at 7,2 km/h	10 mg/h	11 mg/h	47 mg/h 131 " 68 " 90 " 102 " 117 " 79 "	0-80 80-170 170-260 260-350 350-440 440-530 530-575
Mills 1938	241	1 normal	normal		Rothera's test negative	Rothera's test negative	Rothera's test positive	0-180
		1 normal			Rothera's test negative	Rothera's test negative	Rothera's test negative	0-180

Author and Date	Ref.	Subjects	Diet	Exercise	Urinary Ketone Bodies			Time after exercise (minutes)
					Before exercise	On cessation of exercise	During recovery	
Drury 1936-41	78	1 normal	keto-genic	bed rest all day	10,7 g acetone / 24 h			
				normal activities	5,1 g acetone / 24 h			
				2,5 MJ extra work	5,9 g acetone / 24 h			
				6,0 MJ extra work	5,5 g acetone / 24 h			
					Day	Night		
	79	1 normal	keto-genic	bed rest all day	3,6g acetone/12 h	3,5g acetone/12 h		
				normal activities	1,3g acetone/12 h	2,9g acetone/12 h		
				2 hours of tennis	1,2g acetone/12 h	4,8g acetone/12 h		
Sargent 1951	287	troops	normal	day's marching		ketonuria in many cases		
Passmore 1958-60	261	3 trained	12,6MJ for 2 days	16 km walk at 6,7 km/h	0,13mmoles/h	0,24mmoles/h	0,45mmoles/h	0-360
		7 un-trained			0,09mmoles/h	0,12mmoles/h	0,30mmoles/h	0-360
	164	1 un-trained	normal	16 km walk at 6,7 km/h	0 mmoles/h	0 mmoles/h	1,20mmoles/h	300
Pugh 1967-69	268	35 marathon runners	normal	42 km marathon race		no ketonuria		
	269	6 hill walkers	normal	45 km walk in rough country		ketonuria in all subjects		
Johnson 1969-74	178	Boy scouts	normal	walking all day in Derbyshire hills		ketonuria in all subjects		
	68	hill walkers	normal	20 km walk at 4-6 km/h		ketonuria in all subjects		

Author and Date	Ref.	Subjects	Diet	Exercise	Blood Ketone Bodies (mg/100 ml)			Time after exercise (minutes)
					Before exercise	On cessation of exercise	During recovery	
Gemmill 1934	116	3 normal	normal	50 W for 1 hour	0,2	0,2	0,2	60
			low carbohydrate		1,2	1,4	1,9	120
Courtice 1936	69	1 normal	normal	16 km walk at 7,2 km/h	0	0,2	0	120
			slight carbohydrate restriction		0	0	6,3	120
Barnes 1940	30	1 normal	ketogenic	"heavy exercise"	26,4	9,5	16,3	240
Assmussen 1940	15	2 normal	normal	17 km walk up 8,6% gradient at 5,6km/h	0	1,9	4,8	30
Drury 1940-41	79	4 normal	20 h fast	20 minutes running	10,5	6,6		
	80	1 normal	15 h fast	120 minutes walking	1,5	2,5	5,4	60
				160 minutes tennis	6,7	12,8	19,5	100
				5 km run at 16 km/h	15,7	9,6	20,3	210
		rats	normal then 2 day fast	swimming to exhaustion	9,0	8,0	25,0	180
			low protein then 3 day fast		21,0	9,0	32,0 28,0	180 360
Neufeld 1943	247	2 normal	normal	72 W for	"no ketonaemia"			
			ketogenic	3 x 15 minutes (interspersed with 105 minute rest periods) a	6,0	3,0	9,0	105
				b	9,0	7,0	12,0	105
				c	12,0	9,0	14,0 13,0	105 360
Heilesen 1947	138	1 normal	24 h fast	cycling at 176 W for 60 minutes	83,0	120,0	190,0 140,0	15 150
Gammeltoft 1952	114	normal	4-6 day fast	cycling at 59-118 W for 60 minutes	44,0	61,0	65,0 58,0	5 60
Grollman 1954	129	untrained rats	normal	exercise to exhaustion on an exercise wheel	1,56	4,32		
		trained rats			1,77	8,98		
Teräslinna 1966	322	3 normal	normal	10 minutes cycling at 120 W - 240 W	1,21	1,95	1,94 1,25	10 20
Łukawska 1969	213	10 normal teenagers	normal	regular Harvard step test (5 mins.)	2,3	3,9		
		23 trained teenagers			2,9	3,1		
		5 trained teenagers		Harvard step test till exhausted (17-21 minutes)	2,2	3,4		

Author & Date	Ref.	Subjects	Diet	Exercise	Blood Ketone Bodies (mmoles/l)			Time after exercise (minutes)
					Before exercise	On cessation of exercise	During recovery	
Åkerblom 1965	1	7 children	normal	13 minutes cycling at heart rate 170 beats/min.	0,217	0,155	0,252 0,147	150 330
		15 children		45-90 minutes skiing, marching or running	0,178	0,199	0,128 0,200	120 420
Johnson 1970-76	68	3 normal	normal	1½ hours running	<0,1	0,9	2,4	90
	166	18 normal			<0,1	0,8	1,6	90
	169	1 normal			<0,1	0,1	0,4	90
	167	9 normal			<0,1	0,6	1,2	90
	173	8 normal			<0,1	0,3	0,7	90
	68	3 athletes	normal	1½ hours running	<0,1	0,1	0,2	90
	166	9 athletes			<0,1	0,1	0,3	90
	169	1 athlete			<0,1	0,1	0,1	90
	173	5 athletes			<0,1	0,1	0,1	90
	273	6 athletes			0,02	0,06	0,24	105
	171	21 normal	normal	7 km run at 14 km/h			0,16 0,25	60 120
	272	8 normal before training	normal	1 hour running	0,07	0,08	0,37	90
		after training			0,05	0,07	0,10	90
	271	5 normal	normal	20 minutes cycling at heart rate 150-170 beats/minute	0,05	0,05	0,18	150
		4 racing cyclists			0,03	0,03	0,06	150
	168 170	8 normal	normal	30 minutes cycling at 65 W	0,10	0,09	0,17	90
		6 hypo-pituitary			0,06	0,07	0,31	90
	174	9 normal	normal	30 minutes cycling at 80 W	0,06	0,08	0,19	90
		7 acro-megaly			0,08	0,18	0,10	90
	61	6 normal	normal	20 minutes cycling at 100 W	0,04	0,04	0,04	100
		6 alcohol-ics			0,05	0,04	0,18	100
Schmidt 1970	296	14 marathon runners	normal	Deutschen Meister-schaften 1968	0,05	0,32		
Carlson 1971	60	24 normal	normal	cycling for "as long as possible" at heart rate 145-165 beats/minute	0,2	0,2	0,4	60
Houghton 1971	150	normal rats	2 day fast	10 minute run at 0,45 m/s	3,39	1,92		
		trained rats			2,18	1,21		
Eriksson 1971	85	8 normal	normal	60 minutes at 63% $\dot{V}_{O_2}$ max.	0,09	0,11	0,27	60

Author and Date	Ref.	Subjects	Diet	Exercise	Blood Ketone Bodies (mmoles/l)			Time after exercise (minutes)
					Before exercise	On cessation of exercise	During recovery	
Boninsegna 1974	48	rats	36 hr fast	60 minute swim		0,7	1,3	30
Koivisto 1974	194	rats	normal	30 minutes on exercise wheel	0,27	0,59	0,59	120
Winder 1975-79	355	normal rats	normal	0,27 m/s run for 90 min	0,3	1,3	2,1	60
		trained rats	normal	0,27 m/s run for 90 min	0,3	0,9	0,6	60
				0,54 m/s run for 90 min	0,3	0,9	0,9	60
	356	6 normal before training	normal	90 minute cycling at 60% of pretraining $\dot{V}_{O_2}$ max.	0,06	0,16	0,42	60
		after 3wks training					0,14	60
		after 9wks training			0,02		0,08	60
Kuroshima 1975	201	8 rats	normal	30 minute swim	1,61	1,37		
		9 rats	high fat		1,57	1,18		
Askew 1975	14	normal rats	variety of diets	exercise to exhaustion on treadmill	0,40	0,81		
		trained rats			0,49	2,10		
Rennie 1976	275	4 rats	normal	30 minutes on treadmill at 0,35-0,51 m/s up 15-20% gradient	0,23	0,26		
		4 rats	normal + cornoil + heparin before exercise		0,37	0,25		
	274	5 normal	normal	30 minutes cycling at 60% $\dot{V}_{O_2}$ max.	0,09	0,05	0,18 0,11	15 90
Berger 1977	37	6 normal	normal	mild cycling for 180 minutes	0,2	0,6	0,9	30
Norris 1978	250	10 normal	normal	16 km run	0,20	0,45		
Ballasse 1978	25	obese	3 day fast	20 minutes cycling at 50 W			"ketone levels rose slowly with a peak occurring 2-3 hours after cessation of work"	
Zinman 1979	365	7 normal	normal	45 minutes cycling at 50% $\dot{V}_{O_2}$ max.	0,21	0,26	0,37	60
Kemmer 1979	189	4 normal	normal	90 W for 30 minutes	0,21	0,31	0,34	15
Scheele 1979	295	athletes	normal	10 km race	0,193	0,551		
				25 km race	0,240	0,860		
				42 km race	0,244	0,930		

APPENDIX 2

LABORATORY TECHNIQUES

DETERMINATION OF ACETOACETATE AND D-3-HYDROXYBUTYRATE CONCENTRATIONS IN THE BLOOD

The determination of the acetoacetate and D-3-hydroxybutyrate concentrations in the blood were carried out according to the method of Williamson, Mellanby and Krebs (345), using 3-hydroxybutyrate dehydrogenase prepared from Rhodopsuedomonas spheroides (Boehringer Mannheim Gmb, West Germany. Cat. No. 127841).

The enzymatic determination of ketone bodies is specific and allows acetoacetate and 3-hydroxybutyrate to be measured separately. It is also very sensitive, and allows these substances to be determined in the blood of normal postabsorptive subjects (340). Acetone cannot be measured with this method, but it makes up only a few percent of the total concentration of ketone bodies in the blood, and probably has no physiological significance (276,340).

Principle

The method is based on the fact that 3-hydroxybutyrate dehydrogenase catalyses the reaction

$$\text{3-hydroxybutyrate} + NAD^+ \rightleftharpoons \text{acetoacetate} + NADH + H^+$$

The equilibrium constant at 25^oC is $1{,}42 \times 10^{-9}$ (340). To determine the 3-hydroxybutyrate concentration the reaction is made to proceed from left to right, using a ketone trap (hydrazine), and a pH of 8,5 in the reaction mixture. In this way all the 3-hydroxybutyrate is converted to acetoacetate. The rise in absorbance at 340 nm, due to the conversion of NAD^+ to NADH is then an expression of the 3-hydroxybutyrate concentration. When the reaction is made to go from right to left at pH 7,0 the fall in absorbance at 340 nm is proportional to the amount of acetoacetate which has been converted to 3-hydoxybutyrate.

Reagents and Apparatus

Perchloric acid, 0,7 moles/l.

10 ml 70% perchloric acid, $HClO_4$ (E. Merck, Darmstadt, Art. 519) made up to 100 ml with distilled water.

Potassium hydroxide, 4,3 moles/l.

25 g KOH dissolved in 100 ml distilled water.

Phenol red indicator. (The British Drug Houses Ltd. London).

DL-β-hydroxybutyric acid sodium salt, A grade (Calbiochem, San Diego, U.S.A. Cat. No. 3905).

Acetoacetoacetic acid, Lithium salt. Grade II. (Sigma, St. Louis, U.S.A. Cat. No. A8509).

Tris buffer, 0,1 M, pH 8,5.

Dissolve 1,21 g Tris-(hydroxymethyl)-methylamine (AnalaR, BDH Chemicals Ltd., Poole, England. Cat. No. 10315) in 50 ml distilled water. Adjust the pH to 8,5 with 0,2 M HCl, and make up to 100 ml with distilled water. Check the pH.

Hydrazine buffer, pH 8,5.

To 1 ml hydrazine hydrate 99 - 100% (AnalaR, BDH Chemicals Ltd., Poole, England. Cat. No. 10327) add 1,0 M HCl till pH is 8,5. Prepare fresh daily.

Phosphate buffer, 0,1 M, pH 7,0.

Dissolve 13,9 g KH_2PO_4 in 1 litre distilled water; and 17,4 g K_2HPO_4 in 1 litre distilled water. Mix the 2 solutions and check the pH.

NAD

Dissolve 50 mg Nicotinamide adenine dinucleotide (Miles Laboratories (Pty) Ltd., South Africa, Cat. No. 36-299) in 10 ml distilled water. Prepare fresh daily.

NADH

Dissolve 5 mg Nicotinamide adenine dinucleotide, reduced (Miles Laboratories (Pty) Ltd., South Africa. Cat. No. 36-299) in 5 ml distilled water. Prepare fresh daily.

3-hydroxybutyrate dehydrogenase Grade II (Boehringer Mannheim GmbH, West Germany. Cat. No. 127841).

Spectronics 20 spectrophotometer. (Bausch & Lomb, obtained through Laboratory & Scientific Equipment Co. (Pty) Ltd., Cape Town).

Procedure

Ten ml perchloric acid is pipetted in a plastic centrifuge tube standing in an ice tray. To this is added 4 ml of venous blood. The mixture is stirred with a glass rod, and kept in ice till it is centrifuged, to prevent spontaneous decarboxylation of the acetoacetate. The specimen is then centrifuged at 3000 G for 10 minutes, to remove the precipitated protein.

Exactly 10 ml of the supernatant solution is then pipetted into a clean plastic centrifuge tube. A drop of phenol red is added, and enough 4,3 M KOH solution to neutralize the perchloric acid. The volume of KOH solution used is noted. After mixing well the specimen is stored overnight at $-20^{o}C$ to ensure maximal precipitation of $KClO_4$.

The next morning the specimen is thawed and centrifuged at 3000 G for 10 minutes. From this step the analysis procedure for acetoacetate and 3-hydroxybutyrate varies.

Determination of acetoacetate concentration

2 ml of the supernatant is transferred to a glass cuvette, to which are also added 2,8 ml phosphate buffer, and 0,2 ml NADH solution. This mixture has to be mixed extremely well on a

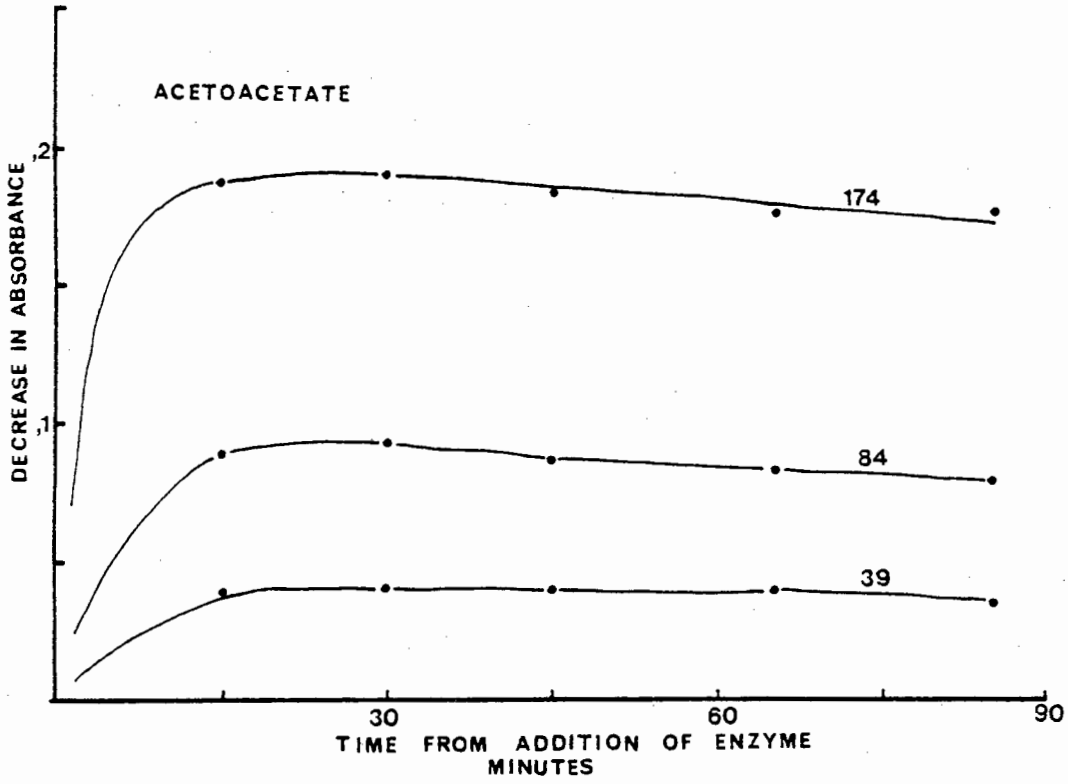

Fig. 34. The changes in absorbance against time of the contents of cuvettes containing different amounts of acetoacetate. The measured concentration of acetoacetate in each cuvette in (nmoles/5 ml) is given on the graph.

vortex mixer to dissolve any $KClO_4$ crystals which may have been transferred from the centrifuge tube.

Absorbance is measured at 340 nm, using distilled water as reference. When the reading is stabilized, the absorbance is noted - A_0.

0,04 ml 3-hydroxybutyrate dehydrogenase enzyme is then added, and the mixture is well stirred on a vortex mixer. After standing for 20 minutes at room temperature, the absorbance is measured at 10 minute intervals, till the fall in absorbance is undetectable (Fig. 34). This reading is noted - A_1.

A blank, prepared from the perchloric acid solution without blood added to it, and a standard, prepared from a solution of

lithium acetoacetate in perchloric acid, are treated in the same manner.

The concentration of acetoacetate in the cuvette is then calculated according to the following formula:

$$\text{acetoacetate } (\mu\text{moles/5 ml}) = \frac{\Delta A \times 5{,}0}{6{,}22 \times 1{,}17}$$

where ΔA is the difference in absorbance ($A_0 - A_1$) of the specimen minus the change in absorbance in the blank; 6,22 is the coefficient of extinction for NADH ($cm^2/\mu mole$); 1,17 is the light path in cm; and 5,0 is the assay volume (ml).

Determination of 3-hydroxybutyrate concentration

1,0 ml of the supernatant is transferred to a glass cuvette, to which is added 3,7 ml Tris buffer, 0,1 ml hydrazine buffer and 0,2 ml NAD solution. The mixture is thoroughly stirred on a vortex mixer, and the absorbance is measured at 340 nm, using distilled water as reference. When the reading is stabilized, the absorbance is noted - A_0.

0,04 ml 3-hydroxybutyrate dehydrogenase is then added to the mixture which is again thoroughly stirred on a vortex mixer. It is then left to stand for 60 minutes at room temperature. Readings of the absorbance are then taken every 10 minutes till the rise in absorbance becomes undetectable (Fig. 35). This reading is noted - A_1. A blank, prepared from the perchloric acid solution without blood added to it, and a standard, prepared from a solution of DL-β-hydroxybutyrate in perchloric acid, are treated in the same way.

The concentration of 3-hydroxybutyrate in the cuvette is then calculated according to the formula:

$$\text{3-hydroxybutyrate } (\mu\text{moles/5 ml}) = \frac{\Delta A \times 5{,}0}{6{,}22 \times 1{,}17}$$

where ΔA is the difference in absorbance ($A_1 - A_0$) of the

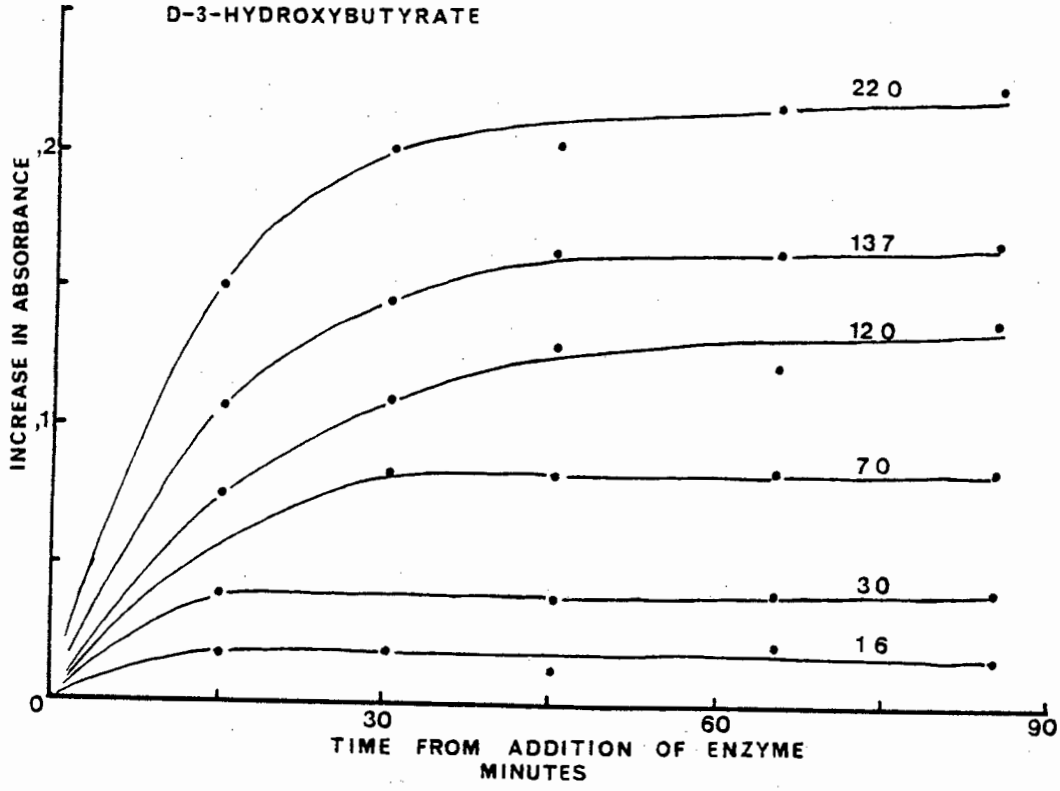

Fig. 35. The changes in absorbance against time of the contents of cuvettes containing different amounts of 3-hydroxybutyrate. The measured concentration of 3-hydroxybutyrate in each cuvette (nmoles/5 ml) is given on the graph.

specimen minus the increase in the absorbance in the blank; 6,22 is the coefficient of extinction for NAD ($cm^2/\mu mole$); 1,17 is the light path in cm; and 5,0 in the assay volume (ml).

Dilution of specimens

The dilution is such that the amount of acetoacetate 3-hydroxybutyrate in the cuvette has to be multiplied by

$$\frac{10 + \text{volume of KOH used (ml)}}{\text{volume of sample in cuvette (ml)}} \times 350$$

to obtain the concentration in 1 litre blood.

If the concentration of ketone bodies in the blood is expected to be high, or if the change in absorbance in the cuvette

exceeds 0,3 units, the neutralized supernatant solution is diluted with the appropriate buffer: Tris in the case of the 3-hydroxybutyrate determination, and phosphate buffer in the case of the acetoacetate determination. Eight-fold dilutions had to be made to read some of the highest blood ketone body concentrations found in this study.

Reproducibility of the analyses

On determination of the acetoacetate concentration in the blood, a coefficient of variation of 15,7% was found on multiple analyses (up to 10 x) of 10 different blood samples with concentrations of acetoacetate of more than 0,1 mmoles/l. For samples containing less than 0,05 mmoles/l the coefficient of variance was 36,1%.

On determination of the 3-hydroxybutyrate concentration in the blood, a coefficient of variation of 7,3% was found on multiple analyses (up to 10 x) of 10 different blood samples, when the concentration in the blood exceeded 0,2 mmoles/l. At concentrations less than 0,1 mmoles/l the coefficient of variation was 17,1%.

Recovery studies

Fresh standard solutions of lithium acetoacetate and DL-3-hydroxybutyrate in 0,7 M perchloric acid were prepared, for each set of analyses. The results of the recovery of acetoacetate and D-3-hydroxybutyrate are illustrated in Figure 36. The mean recovery of D-3-hydroxybutyrate was 78,7% (± SD 5,5). The mean recovery of acetoacetate was 75,5% (± SD 15,4). Both the 3-hydroxybutyrate and acetoacetate assays showed a fall off in recovery with high concentrations of these substances in the cuvette.

The reason for the low recoveries is not known, but they were consistent, and the readings of the blood ketone body concentrations in the text are the unadjusted values calculated directly from the changes in absorbance. There is therefore

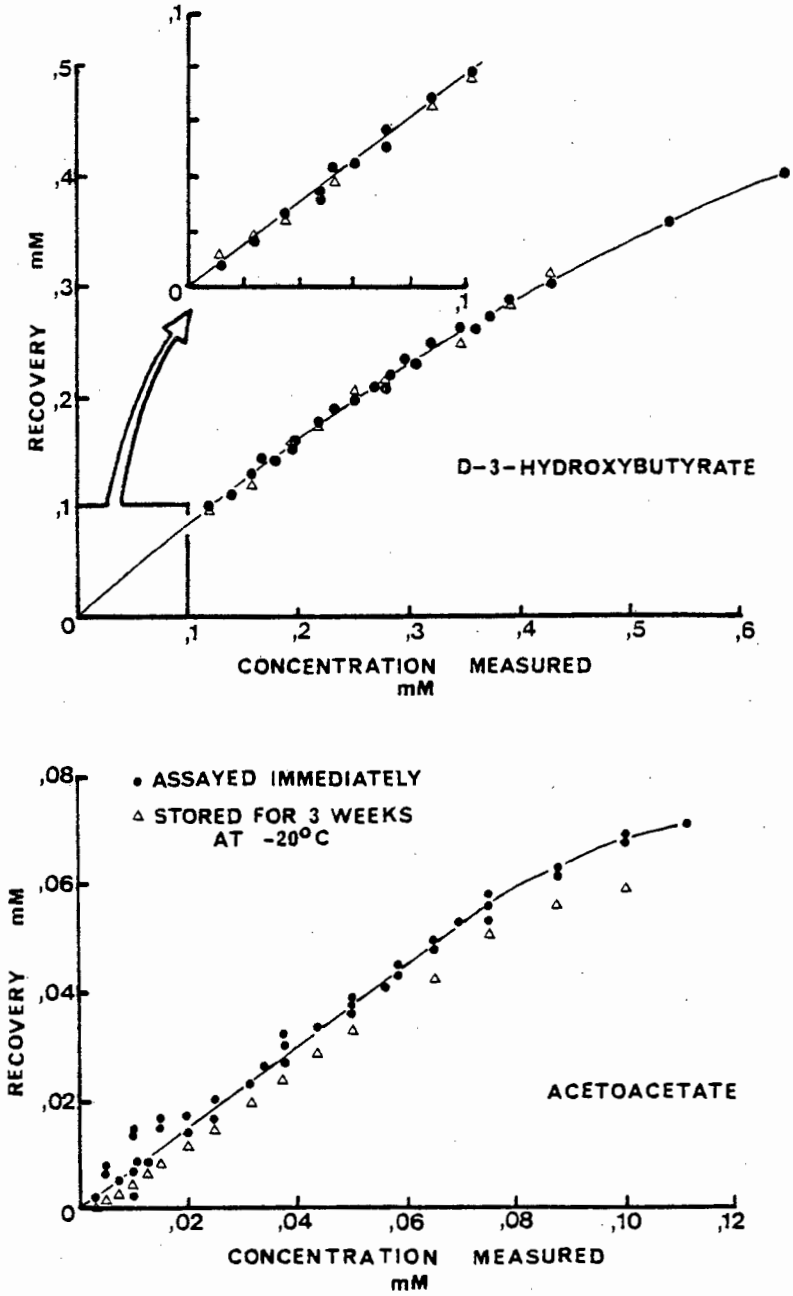

Fig. 36. The recovery of 3-hydroxybutyrate and acetoacetate added to 0,7 M perchloric acid.

a possibility that all our readings are about 20 - 25% lower than the actual blood ketone body concentrations.

Stability of the ketone bodies

When the standard solutions were kept at -20°C for 3 weeks, there was no difference in the recovery of 3-hydroxybutyrate, but the recovery of acetoacetate was reduced by about 0,003 mmoles/l, at all concentrations up to 0,075 mmoles/l (Fig. 36).

This was probably due to an error in the blank, rather than to a loss of acetoacetate from the solution. The blood samples were always analysed within 24 hours.

DETERMINATION OF L-LACTATE CONCENTRATIONS IN THE BLOOD

The blood L-lactate concentrations were determined using the Boehringer Mannheim Biochemica Test Combination (Cat. No. 15972).

Principle

The determination of L-lactate in the blood depends on the fact that lactate dehydrogenase catalyses the reaction:

$$\text{lactate} + NAD^+ \rightleftharpoons \text{pyruvate} + NADH + H^+$$

The equilibrium lies to the left, and if the reaction is therefore to proceed from left to right, pyruvate must be removed from the reaction mixture by means of a ketone trap (hydrazine), at pH 9,0.

The rise in absorbance at 340 nm is due to the conversion of NAD to NADH, which is, in turn, proportional to the amount of lactate converted to pyruvate.

Reagents and Apparatus

Perchloric acid, 0,7 M

10 ml 70% perchloric acid (Merck, Darmstadt. Art. 519) is diluted up to 100 ml with distilled water.

The Test Combination Kit contains:

Glycine-hydrazine buffer. The contents of the bottle are diluted in 50 ml distilled water.

NAD (nicotinamide adenine dinucleotide), of which 15 mg is dissolved in 5 ml distilled water. Prepare fresh daily.

Lactate dehydrogenase.

Standard L-lactate solution 1N.

Spectronics 20 spectrophotometer (Bausch & Lomb, obtained through Laboratory & Scientific Equipment Co. (Pty) Ltd., Cape Town).

Procedure

10 ml 0,7 M perchloric acid solution is pipetted into a plastic centrifuge tube, To this is added 4 ml venous blood, and the mixture is stirred with a glass rod. It is then centrifuged at 3000 G for 10 minutes to remove the precipitated protein.

Exactly 0,1 ml of the supernatant solution is then transferred to a glass cuvette, to which is also added 4,4 ml glycine-hydrazine buffer solution, and 0,5 ml NAD solution. This mixture is thoroughly stirred.

Absorbance is measured at 340 nm, using distilled water as reference. When the reading is stabilized, the absorbance is noted - A_o.

0,02 ml of lactate dehydrogenase enzyme solution is then added, and the mixture is stirred on a vortex mixer. After standing for 20 minutes at room temperature, the absorbance is again measured every 10 minutes, till the increase in absorbance is undetectable. This reading is noted - A_1.

A blank, prepared from the 0,7 M perchloric acid solution without blood added to it, and a standard, prepared from the 1 N L-lactate solution, are treated in the same way.

The concentration of L-lactate in the cuvette is then calculated according to the following formula:

$$\text{lactate } (\mu\text{moles/5 ml}) = \frac{\Delta A \times 5{,}0}{6{,}22 \times 1{,}17}$$

where ΔA is the difference in absorbance ($A_1 - A_o$) of the specimen minus the change in absorbance in the blank; 6,22 is the coefficient of extinction for NAD ($cm^2/\mu mole$); 1,17 is the light path in cm; and 5,0 is the assay volume in ml.

Dilution of specimen

The dilution factor is such that the amount of lactate in the cuvette has to be multiplied by 35000 to obtain the concentration

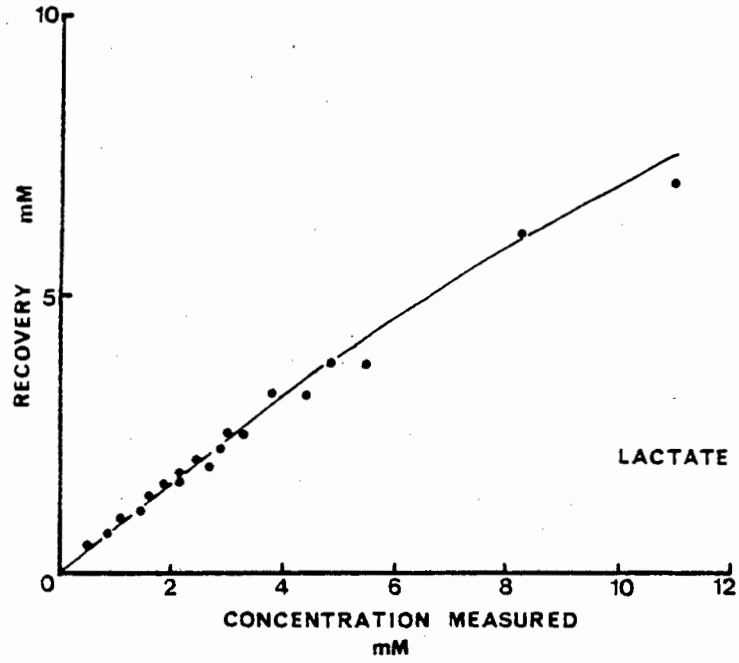

Fig. 37. The recovery of L-lactate added to 0,7 M Perchloric acid.

of lactate in 1 litre of blood.

If the concentration of lactate in the blood is expected to be high, e.g. after exercise, the supernatant is diluted 2-fold in glycine-hydrazine buffer before being added to the cuvette.

Reproducibility of the analysis

The coefficient of variation for the assay was 15,2% for multiple analyses (up to 10 x) of 10 different blood samples with lactate concentrations of less than 1 mmole/l.

Recovery studies

Fresh standard solutions of L-lactate in 0,7 M perchloric acid were prepared for each set of analyses. The results of the recovery of L-lactate are illustrated in Figure 37. The mean recovery of L-lactate was 80,4 (± SD 9,7)% for concentrations

up to 8,0 mmoles/l. The concentrations of L-lactate in the blood reported in the text are the unadjusted values calculated directly from the changes in absorbance.

THE DETERMINATION OF LONG-CHAIN FREE FATTY ACID CONCENTRATIONS IN THE BLOOD

The long-chain free fatty acid concentrations in the serum were determined according to the method of Dole and Meinertz (77).

Principle

Long-chain non-esterified fatty acids are extracted from the serum with a two-phase heptane-isopropyl alcohol-water system, that provides sufficient analytical specificity for the determination of these lipids under usual circumstances. If exceptionally high levels of ketone bodies or acetic or lactic acid are present, a second extraction eliminates any interference (77).

The concentration of the free fatty acids in the upper phase is determined by titration with carbonate-free NaOH, using Nile Blue as indicator.

Reagents

Dole's Solution.

To 40 parts iso-propyl alcohol (Hopkin & Williams Cat. No. 721600) add 10 parts n-heptane (May & Baker Ltd., Dagenham, England) and 1 part 1 N H_2SO_4 (AE & CI).

n-Heptane (May & Baker Ltd., Dagenham, England)

Palmitic Acid Standard, 1 mM.

Dissolve 0,768 g crystalline palmitic acid (BDH, Poole, England. Cat. No. 29433) in 500 ml n-heptane. Dilute this stock solution 1:6 with n-heptane for the titration.

Nile Blue Indicator.

Dissolve 100 mg dry Nile Blue (BDH, Poole, England. Cat.

No. 34059) in 100 ml CO_2-free distilled water. Filter and dilute to 500 ml. Wash with iso-octane until the washings are clear. Dilute this stock solution 1:5 with absolute alcohol B.P., and bubble N_2 through for 10 minutes before use in titration. Check the pH, that it is 8, and adjust if necessary with 0,1 M NaOH.

NaOH, 0,01 M.

Dissolve 1 ampoule 'Volucon' Sodium Hydroxide (May & Baker, Dagenham, England) in 1 litre distilled water. Dilute this stock solution 1:10 in distilled water just before use.

Procedure

Venous blood is allowed to clot at 0°C and is then centrifuged to obtain serum, which is immediately frozen at -20°C till needed, to prevent lipolysis of the triglycerides in the serum.

Specimen

To 0,6 ml thawed serum in a glass stoppered tube is added 3,0 ml Dole's solution. Shake the mixture for 3 minutes, and allow to stand for about 5 minutes (the exact time is not critical).

Standard

To 0,6 ml palmitic acid standard (1 mM) in a glass stoppered tube add 3,0 ml Dole's solution, and shake for 3 minutes. Then allow to stand for 5 minutes.

Blank

To 0,6 ml distilled water in a glass stoppered tube add 3,0 ml Dole's solution and shake for 3 minutes. Allow to stand for 5 minutes.

To all the tubes now add 1,8 ml n-heptane and 1,2 ml distilled water, and shake again for 5 minutes. Allow to stand for at

least 30 minutes.

Transfer 1 ml of the upper phase to a clean glass tube and add 1 ml Nile Blue indicator. Titrate against 0,01 M NaOH, bubbling nitrogen gas through from the bottom of the tube to ensure constant mixing. The tube is mounted in front of a white sheet of paper to see the colour change in the mixture. The end point (a pink colour) is read by stopping the nitrogen flow momentarily to permit separation of the phases. It is quite sharp, and usually reproducible to about 1 µl of NaOH solution.

Calculation

The concentration of long-chain free fatty acids in the serum is calculated from the volumes of NaOH used, as follows:

$$\text{serum free fatty acid concentration (mmoles/l)} = \frac{\text{specimen - blank}}{\text{standard - blank}} \times \text{concentration of palmitate in standard}$$

Reproducibility of the analysis

The coefficient of variation for the analysis was 13,9% for multiple (up to 10 x) analyses of different blood samples.

DETERMINATION OF D-GLUCOSE CONCENTRATIONS IN THE SERUM

The serum D-glucose concentration was determined using the Boehringer Mannheim GOD-PAP Glucose Kit (Cat. No. 166391) on a Technicon Autoanalyser.

Principle

The method is based on the fact that glucose oxidase (obtained from Penicillium notatum) catalyses the reaction:

$$\text{glucose} + O_2 + H_2O \longrightarrow \text{gluconate} + H_2O_2$$

The hydrogen peroxide produced in the reaction is then assayed using peroxidase (obtained from horse radish) to bring about a colour change in the 4-aminophenazone:

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \longrightarrow$$

$$\text{4-(p-benzoquinone-mono-imino)phenazone} + 4H_2O$$

The change in absorbance at 500 - 520 nm is then proportional to the glucose concentration in the test solution.

Reagents and Apparatus

The Glucose GOD-PAP Test Kit contains:

Bottle 1: Buffer/enzymes/4-aminophenazone.
Bottle 2: Phenol.
Dissolve the contents of bottle 1 in 200 ml redistilled water and add the contents of bottle 2. Store this reagent mixture in a dark bottle at +4°C.

URAC deproteinizing solution. (Boehringer Mannheim. Cat. No. 125415).

Hyland Q-PAK Chemistry Control Serum (Hyland, Costa Mesa, Ca., U.S.A. Cat. No. 045-030).

Technicon Autoanalyser II.

Procedure

Venous blood is centrifuged immediately after withdrawal from the subject. The plasma is allowed to clot, and is then centrifuged again to obtain serum.

To 0,1 ml serum is added 1 ml URAC solution and the mixture is centrifuged to remove the precipitated protein. This is done within 4 hours of taking the blood. Use the supernatant for the assay.

The Hyland Control Serum ('Standard') is treated in the same way. A 'blank' is prepared from distilled water and the URAC reagent.

The autoanalyser mixes 1 part of the supernatant with 10 parts of the reagent mixture. This is incubated for 10 minutes at $37^{o}C$, and the absorbance is then measured at 500 - 520 nm (A).

Calculation

The concentration of glucose in the serum is calculated from the following formula:

$$\text{serum glucose concentration (mmoles/l)} = \frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times \text{glucose concentration in standard}$$

Reproducibility of the analysis

The coefficient of variation for the analysis was 1,5% for multiple (up to 10 x) analyses of different blood samples.

THE DETERMINATION OF THE SERUM IMMUNOREACTIVE INSULIN AND GROWTH HORMONE CONCENTRATIONS AND PLASMA IMMUNOREACTIVE GLUCAGON CONCENTRATIONS

The concentrations of these hormones in the serum and plasma were determined by radioimmunoassay. The serum immunoreactive insulin and growth hormone concentrations were assayed using the CEA SORIN (International CIS, 2 Rue Stephenson, 78181 St. Quentin Yvelines Cedex, France) radioimmunoassay kits, reference INSIK-1 and HGHK respectively. The procedures followed for the determination of these 2 hormones were exactly as described in the instructions which accompany the kits, and will therefore not be reiterated here. There was no kit for the immunoreactive glucagon determination, and the procedure is therefore described here as an example of the radioimmunoassay technique.

Principle

In general, the radioimmunoassay is described by the following formula:

$$\begin{matrix} \text{antigen (standard or endogenous)} \\ + \\ {}^{125}\text{I antigen} \end{matrix} + \text{antibody} \rightleftharpoons \begin{matrix} \text{antigen-antibody} \\ + \\ {}^{125}\text{I antigen-antibody} \end{matrix}$$

The unlabelled antigen competes with the labelled antigen for a limited number of antibody binding sites, thereby reducing the amount of labelled antigen bound to the antibody. The level of radioactivity bound is therefore inversely related to the concentration of antigen in the subject's sample, or in the standard.

After adequate incubation, the bound and free fractions are separated and the radioactivity quantitated in a scintillation gamma counter adjusted for maximum efficiency for measurement of the radioactive tracer.

Finally a standard curve is plotted and the concentration of the antigen in the subject's sample is determined from the standard curve.

Reagents and Apparatus

Glycine diluent (0,2 M glycine)

15,01 g glycine and 5,85 g NaCl are dissolved in about 700 ml distilled water. Adjust pH to 8,8 with 1 M NaOH. Add 12,5 ml 20% human serum albumin, 10 ml normal rabbit serum, and 50 ml Midran (Novo Industries, Johannesburg. Reg. No. H/32/197). Adjust volume to 1 litre with distilled water.

^{125}I-Glucagon

5 µg porcine glucagon (Eli Lilly, Indianapolis, Ind., U.S.A. Lot No. 258-V016-235) is labelled by the oxidation-reduction method of Hunter and Greenwood (366), and purified on a QAE-Sephadex A 25 anion exchange column according to the method of Jørgensen and Larsen (367).

Antibody

The pancreatic specific antibody 30 K was obtained from Roger H. Unger (University of Texas, South Western Medical School, Dallas, Texas, U.S.A.). Dilute to a final dilution of 1:250 000.

Charcoal-Dextran suspension

15,01 g glycine and 5,85 g NaCl are dissolved in about 700 ml distilled water, and the pH is adjusted to 8,8 with 1 M NaOH. To this is added 5 g activated charcoal (Sigma, St. Louis, U.S.A. Cat. No. 65C-0171) and 2,5 g Dextran T 70 (Pharmacia Fine Chemicals, Uppsala, Sweden. Cat. No. 1730). Adjust volume to 1 litre with distilled water.

Autogamma Scintillation Spectrometer (Packard).

Procedure

0,25 ml Midran (Novo Industries, Johannesburg. Reg. No. H/H/32/197) and a drop (50 IU) of heparin (Labethica, Reg. No. J/8.2/405) are added to a 5 ml glass tube. 2,5 ml venous blood is added to this mixture, stirred by shaking and immediately chilled to 0°C. It is then centrifuged to remove the red blood cells.

The separated plasma is then stored at -20°C until needed.

Specimen

To 200 µl of plasma in a 5 ml cuvette add:

600 µl	glycine diluent
100 µl	antibody solution
100 µl	^{125}I-glucagon solution
1,00 ml	final volume

Vortex mix.

Standards

Standards are prepared from porcine glucagon (Eli Lilly, Indianapolis, Ind. U.S.A. Lot No. 258-V016-235) in glycine diluent. A stock solution of 0,01 mg/ml is stored at -20°C. On the day of the test this is serially diluted in glycine diluent to give standard solutions of 2500, 1000, 500, 250, 100, 50 and 25 pg/ml, using a MicroMedic Diluter.

To 200 µl of each standard solution in a 5 ml cuvette add:

600 µl	glycine diluent
100 µl	antibody solution
100 µl	^{125}I-glucagon solution
1,00 ml	final volume

Vortex mix.

Non-specific Binding

Total charcoal absorption of the labelled antigen is measured by omitting the antibody, standards or the sample plasma from the cuvette. Non-specific charcoal absorption of the subject's plasma is measured by omitting the antibody:

To 800 µl glycine diluent in a 5 ml cuvette add:
100 µl antibody solution
100 µl ^{125}I-glucagon solution
1,00 ml final volume

To 200 µl of plasma in a 5 ml cuvette add:
700 µl glycine diluent
100 µl ^{125}I-glucagon solution
1,00 ml final solution

Vortex mix.

Incubate all the cuvettes for 3 days at +4°C.

Then add 0,2 ml normal horse serum to the standards and tubes without plasma; and add 0,2 ml glycine diluent to the cuvettes containing the plasma samples. Vortex mix. Add 0,5 ml charcoal-dextran suspension, and vortex mix again.

Incubate at +4°C for 20 minutes, and then centrifuge at the same temperature for 15 minutes at 2000 G.

Carefully decant the supernatant; avoid disturbing the precipitate.

Measure the radioactivity of the precipitate (unbound ^{125}I-glucagon) in all the cuvettes on the gamma scintillation counter for 10 minutes or for 10 000 counts.

Calculation

The following calculation is performed on each sample and standard:

$$100 - \frac{\text{unbound } ^{125}\text{I-glucagon} \times 100}{\text{total non-specific charcoal absorption}} \%$$

as an expression of the percentage binding which has taken place. The percentage binding is plotted against the concentration of glucagon in the standards on log - linear paper to construct a calibration curve, from which the concentration of glucagon in the unknown is obtained by interpolation.

Reproducibility

The coefficient of variation for the immunoreactive glucagon estimation was 12,7% for 10 readings of 2 different blood samples.

The coefficient of variation for the immunoreactive growth hormone estimation was 9,6% for 10 readings of a single blood sample.

The coefficient of variation for the immunoreactive insulin estimation was 7,1% for 10 readings of 2 different blood samples.

STATISTICAL METHODS

The data presented in Appendix 3 were analysed on a Hewlett-Packard 9815A Computer:

mean $(\bar{x})$ $= \frac{\Sigma x}{n}$

standard deviation (SD) $= \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}}$

standard error of the mean (SEM) $= \frac{SD}{\sqrt{n}}$

Except in the tables of Appendix 3, the term 'range' is not used in the usual statistical sense. When the 'range' is given in the text, the highest and lowest values are reported, and not the difference between them.

The hypothesis that the mean difference of the population was zero, was tested by Student's t test. Unless stated otherwise, the one-tail, unpaired Student's t test was used. If the probability that the findings were due to chance, was less than 5%, the result is reported as $P < 0,05$. Probability values of less than 5% ($P < 0,05$) are considered as probably significant; $P < 0,01$ as significant, and $P < 0,001$ as highly significant.

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{(SD_1)^2}{n_1} + \frac{(SD_2)^2}{n_2}}}$$

Significance limits of the Student distribution were obtained from 'Scientific Tables' Ed. K. Diem and C. Lenter. Ciba Geigy, Basle (1970) p. 32-33.

APPENDIX 3

TABLES OF RESULTS

KEY

Ketones	= blood ketone body concentration (acetoacetate + 3-hydroxybutyrate) (mmoles/l).
3HB/AA	= 3-hydroxybutyrate/acetoacetate molar ratio in the blood.
Lactate	= blood lactate concentration (mmoles/l).
Heart rate	= beats/minute.
Glucose	= serum glucose concentration (mmoles/l).
FFA (free fatty acids)	= serum long-chain free fatty acid concentration (mmoles/l).
IRI (insulin)	= serum immunoreactive insulin concentration (μU/ml).
IRG (glucagon)	= plasma immunoreactive glucagon concentration (pg/ml).
HGH (growth hormone)	= serum immunoreactive human growth hormone concentration (ng/ml).
$\bar{x}$	= mean.
SD	= standard deviation.
SEM	= standard error of the mean.

CONTROLS

NON-ATHLETES December - March

	Age yrs	Height cm	Mass kg		07h30	08h00	09h30	10h30	13h00	17h00
				Lactate	1,29	0,64	0,45		0,78	0,36
CJ	18	175	55	Ketones	,045	,076	,053		,072	,065
				3HB/AA	2,21	1,92	1,41		2,60	1,95
				Lactate	1,07	0,35	0,62	1,88	0,95	1,86
JM	18	169		Ketones	,111	,094	,048	,065	,026	,100
				3HB/AA	2,58	3,70	1,82	2,25	12,0	2,03
				Lactate	0,61	0,58	0,43	0,54	0,47	0,64
JK	37	178	65	Ketones	,097	,119	,261	,220	,334	,192
				3HB/AA	1,78	2,22	3,83	5,29	4,39	3,68
				Lactate	1,13	0,92	0,88		0,39	0,37
HR	19	183	91	Ketones	0,53	,048	,056		,147	,086
				3HB/AA	7,83	1,18	1,80		2,34	1,97
				Lactate	1,13	0,98	2,28		0,36	2,18
IK	19	178	58	Ketones	,062	,084	,029		,057	,055
				3HB/AA	3,13	0,91	1,90		1,38	0,77
				Lactate	1,07	0,85	0,60		0,68	1,28
AF	19	172	60	Ketones	,076	,023	,032		,018	,091
				3HB/AA	1,38	2,83	1,46		2,00	2,78
									April - September	
				Lactate	1,92	1,86	0,54	1,30	0,12	0,43
AH	19	178	77	Ketones	,068	,013	,016	,037	,094	,375
				3HB/AA	0,45	0,08	0,07	6,40	1,47	3,03
				Lactate	0,99	0,80	0,37	2,12	0,10	0,47
KC	20	180	73	Ketones	,039	,012	,017	,044	,012	,190
				3HB/AA	1,60	11,0	16,0	0,57	11,0	2,33
				Lactate	1,38	0,43	0,39	0,68	1,03	0,87
AK	19	167	69	Ketones	,035	,043	,038	,048	,090	,260
				3HB/AA	1,50	0,23	1,00	1,53	1,00	1,83
				Lactate	0,87	1,05	0,53	0,76	1,11	1,42
PR	18	170	63	Ketones	,061	,041	,025	,038	,064	,141
				3HB/AA	5,78	1,41	24,0	1,24	2,05	4,88
				Lactate	0,71	0,70	0,62		0,66	0,86
ED	19	178	69	Ketones	0,22	,031	,032		,096	,154
				3HB/AA	2,14	1,58	1,13		1,67	3,16

NON-ATHLETES (Cont.)

	Age yrs	Height cm	Mass kg		07h30	08h00	09h30	10h30	13h00	17h00
				Lactate	0,45	0,93	0,85	1,49	1,01	0,80
BM	19	175	74	Ketones	,039	,100	,047	,033	,130	,226
				3HB/AA	0,07	0,52	1,24	32,0	1,46	1,28
				Lactate	2,93	2,36	1,72		2,48	1,20
RW	19	172	67	Ketones	,021	,039	,025		,030	,212
				3HB/AA	2,00	2,00	3,17		2,75	3,33
				Lactate	1,42	0,71	0,54		1,14	0,89
RV	19	184	66	Ketones	,095	,045	,035		,045	,082
				3HB/AA	3,13	1,14	1,69		2,21	0,55
				Lactate	0,89	0,95	0,76	0,66	0,74	0,14
HN	19	181	76	Ketones	,025	,076	,073	,100	,077	,061
				3HB/AA	2,13	4,43	1,92	2,23	1,83	3,69
				Lactate	2,09	0,41	0,41		0,71	0,35
KS	19	177	70	Ketones	,079	0,04	,043		,163	,162
				3HB/AA	0,41	4,71	1,26		2,33	5,23

ATHLETES

January - March

	Age yrs	Height cm	Mass kg		07h30	08h00	09h30	10h30	13h00	17h00
				Lactate						
DC	24	176	67	Ketones	0,12	,123	,134	,216	,128	,389
				3HB/AA	1,55	2,15	1,58	2,27	2,28	1,99
				Lactate						
TN	29	187	77	Ketones	,110	,097	,067	,061	,046	,294
				3HB/AA	0,75	0,94	0,81	0,85	2,09	1,75
				Lactate	0,64	1,71	1,14	0,91	0,42	0,21
MT	20	186		Ketones	,088	,048	,056	,105	,133	,063
				3HB/AA	2,38	5,00	3,31	3,04	3,75	2,15
				Lactate	0,54	0,64	0,33		0,33	0,85
RS	23	177	75	Ketones	,025	,017	,019		,086	,040
				3HB/AA	1,06	0,70	0,58		4,73	3,44
				Lactate	0,37	0,33	0,52	0,43	0,23	0,31
SH	20	176	71	Ketones	,105	,047	,028	,073	,067	,069
				3HB/AA	2,50	2,62	13,0	0,46	1,68	1,56
									April - September	
				Lactate	1,40	1,06	0,77	0,98	0,46	0,58
PS	19	172	66	Ketones	,038	,096	,044	,075	,167	,129
				3HB/AA	2,17	3,80	4,50	2,75	5,96	3,78
				Lactate	1,15	0,58	0,93	0,89	1,61	0,58
NB	19	178	74	Ketones	,109	0,50	,022	,063	,098	,164
				3HB/AA	2,30	1,08	0,83	2,50	3,08	3,32
				Lactate	0,74	0,73	0,50	1,16	0,71	0,62
MMc	19	177	61	Ketones	,045	,003	,025	,007	,011	0,37
				3HB/AA	0,55	2,00	0,39	2,50	10,0	5,17
				Lactate	0,84	0,62	0,95	1,34	0,41	1,24
DV	19	177	61	Ketones	,077	,085	,026	,096	,176	,207
				3HB/AA	0,45	1,02	0,04	1,00	3,19	1,20
				Lactate	0,91	0,26	0,20	0,42	0,67	0,26
TM	26	186	76	Ketones	,054	,055	,031	,083	,056	,022
				3HB/AA	1,35	2,24	2,10	2,61	5,22	21,0
				Lactate	0,89	0,59	0,48	0,53	0,53	1,04
SO	20	182	74	Ketones	,051	,007	,015	,015	,022	,139
				3HB/AA	0,46	0,40	0,88	14,0	1,75	2,56

ATHLETES (Cont.)

	Age yrs	Height cm	Mass kg		07h30	08h00	09h30	10h30	13h00	17h00
				Lactate	1,04	1,73	0,93	1,09	1,38	0,45
DY	19	180	98	Ketones	,085	,061	,006	,004	,041	,026
				3HB/AA	0,60	0,53	5,00	3,00	0,46	2,71
				Lactate	0,96	0,76	0,49	0,99	0,24	0,89
MB	19	179	79	Ketones	,067	,027	,006	,043	,040	,156
				3HB/AA	0,02	8,00	5,00	0,23	0,90	1,29
				Lactate	0,43	0,47	0,78	0,58	0,93	1,07
CH	19	192	101	Ketones	,031	,009	,039	,036	,044	,279
				3HB/AA	0,11	8,00	0,03	0,03	0,91	5,98
				Lactate	0,62	0,27	0,74	0,70	0,31	0,37
MM	19	177	61	Ketones	,056	,063	,056	,065	,050	,131
				3HB/AA	3,31	4,73	3,31	4,00	6,14	2,97
				Lactate	1,14	0,64	1,11		1,15	1,40
KP	19	185	75	Ketones	,035	,037	0,08		,066	,131
				3HB/AA	2,18	2,36	1,29		1,54	2,45
				Lactate	0,32	0,62	0,14		0,27	0,70
PB	19	177	75	Ketones	,029	,048	,022		,005	,019
				3HB/AA	2,63	0,65	21,0		0,25	2,80
				Lactate	1,55	0,35	0,45		1,20	1,15
KO	18	188	86	Ketones	,028	,013	,026		,079	,142
				3HB/AA	1,33	12,0	0,63		2,29	1,68

COMPLETE REST Room temperature 17-19°C

	Age yrs	Height cm	Mass kg		08h30	09h30	10h30	13h00	17h00
NON-ATHLETES									
DQ	27	183	72	Lactate	1,26	0,93	0,58	0,80	0,43
				Total ketones	,083	,079	,066	,079	,058
				3HB/AA	0,54	0,58	0,80	0,25	0,81
DC	18	177	65	Lactate	0,58	0,72	2,62	1,11	0,66
				Total ketones	,065	,069	,059	,059	,106
				3HB/AA	0,32	0,30	0,55	2,69	1,41
DW				Lactate	0,39	0,16	0,23	0,43	1,16
				Total ketones	,111	,064	,092	,286	,702
				3HB/AA	2,00	0,60	29,7	3,27	2,12
AS	20	165	64	Lactate	1,03	0,59	0,83	1,22	0,52
				Total ketones	,038	,030	,023	,045	,127
				3HB/AA	0,90	0,50	0,15	0,50	1,76
ATHLETES									
PBo				Lactate	0,29	2,64	0,29	0,64	0,70
				Total ketones	,028	,008	,058	,013	,113
				3HB/AA	6,00	8,00	0,23	13,0	2,05
JB	19	179	79	Lactate	0,31	0,56	0,52	0,84	0,37
				Total ketones	,094	,072	,109	,136	,359
				3HB/AA	0,81	1,40	0,79	1,96	1,56
JH	19	192	101	Lactate	0,43	0,43	0,35	0,48	0,72
				Total ketones	,051	,037	,028	,048	,091
				3HB/AA	0,59	2,36	0,03	1,00	1,39
DY	19	180	89	Lactate	0,76	0,37	0,82	0,52	0,35
				Total ketones	,039	,052	,063	,097	,051
				3HB/AA	2,25	0,02	0,70	0,23	2,00
DB	22	178	69	Lactate	0,31	1,05	1,79	0,47	0,43
				Total ketones	,101	,194	,190	,207	,446
				3HB/AA	(100)	1,69	0,39	0,50	1,34
AP	22	183	72	Lactate	0,82	0,74	0,43	0,72	0,83
				Total ketones	,080	,106	,133	,175	,294
				3HB/AA	1,67	0,54	0,46	0,43	1,19

SWIMMING WITH AND WITHOUT BREATHHOLDING

CONTROL

NON-ATHLETES

	Age yrs	Height cm	Mass kg		07h30	08h00	09h30	10h30	13h00	17h00
				Lactate	1,92	1,86	0,54	1,30	0,12	0,43
AH	19	178	77	Ketones	,068	,013	,016	,037	,094	,375
				3HB/AA	0,45	0,08	0,07	6,40	1,47	3,03
				Lactate	0,99	0,80	0,37	2,12	0,10	0,47
KC	20	180	73	Ketones	,039	,012	,017	,044	,012	,190
				3HB/AA	1,60	11,0	16,0	0,57	11,0	2,33
				Lactate	1,38	0,43	0,39	0,68	1,03	0,87
AK	19	167	69	Ketones	,035	,043	,038	,048	,090	,260
				3HB/AA	1,50	0,23	1,00	1,53	1,00	1,83
				Lactate	0,87	1,05	0,53	0,76	1,11	1,42
PR	18	170	63	Ketones	,061	,041	,025	,038	,064	,141
				3HB/AA	5,78	1,41	24,0	1,24	2,05	4,88
				Lactate	0,45	0,93	0,85	1,49	1,01	0,80
BM	19	175	74	Ketone	,039	,100	,047	,033	,130	,226
				3HB/AA	0,07	0,52	1,24	32,0	1,46	1,28
				Lactate	0,89	0,95	0,76	0,66	0,74	0,14
HN	19	181	76	Ketone	,025	,076	,073	,100	,077	,061
				3HB/AA	2,13	4,43	1,92	2,23	1,83	3,69

NON-ATHLETES

Swim without breathholding
Swim with breathholding

↓ SWIM

	Age yrs	Height cm	Mass kg		07h30	08h15	09h30	10h30	13h00	17h00
ES				Lactate	1,40	2,23	1,40	0,49	1,36	0
				Ketones	,052	,055	,031	,078	,114	,205
				3HB/AA	3,72	2,24	0,48	3,87	2,00	8,76
				Lactate	*3,22*	*4,29*	*0,95*	*0,85*	*0,46*	*0,60*
				Ketones	*,035*	*,026*	*,010*	*,019*	*,129*	*,425*
				3HB/AA	*1,50*	*26,0*	*10,0*	*19,0*	*3,96*	*3,08*
KP	18	178	74	Lactate	0,99	1,67	0,64	2,47	0,95	0,56
				Ketones	,029	,026	,022	,022	,019	,099
				3HB/AA	2,60	26,0	10,5	1,75	2,80	2,19
				Lactate	*2,43*	*2,52*	*0,80*	*1,07*	*0,74*	*1,30*
				Ketones	*,034*	*0,06*	*,029*	*,058*	*,041*	*,181*
				3HB/AA	*0,17*	*0,46*	*0,03*	*0,66*	*0,41*	*2,07*
JV	18	176	64	Lactate	1,92	4,52	1,26	1,71	1,34	1,36
				Ketones	,031	,028	0,04	,034	,032	,178
				3HB/AA	9,33	6,00	4,71	3,89	1,91	2,12
				Lactate	*1,46*	*4,33*	*0,76*	*1,69*	*1,51*	*1,51*
				Ketones	*,068*	*,053*	*,080*	*,044*	*,128*	*,276*
				3HB/AA	*0,45*	*0,29*	*0,70*	*0,91*	*2,12*	*2,94*
BMt	18	181	70	Lactate	0,84	1,81		1,45		1,08
				Ketones	,056	,066		,116		,238
				3HB/AA	0,40	0,01		0,46		2,01
				Lactate	*0,72*		*1,03*	*0,56*	*0,27*	*0,82*
				Ketones	*0*		*,045*	*,006*	*,247*	*,363*
				3HB/AA	-		*0,06*	*6,00*	*24,0*	*2,00*
MC	20	180	73	Lactate	1,16	1,45	0,80	1,20	0	1,32
				Ketones	,052	,084	,066	,198	,185	,447
				3HB/AA	1,00	0,06	0,65	2,00	(185)	1,59
				Lactate	*0,72*		*0,72*	*0,83*	*0,51*	*0,59*
				Ketones	*,252*		*,075*	*,195*	*,242*	*,605*
				3HB/AA	*1,80*		*3,98*	*1,60*	*(242)*	*5,67*

↓SWIM

	Age yrs	Height cm	Mass kg		07h30	08h15	08h30	10h30	13h00	17h00
JT	29	185	78	Lactate	1,38	5,13	1,07	0,74	1,34	1,36
				Ketones	,024	,031	,010	,029	,095	,311
				3HB/AA	24,0	31,0	10,0	29,0	39,0	3,94
				Lactate	*0,33*	*4,14*	*0,87*	*2,10*	*0,23*	*0,66*
				Ketones	*,061*	*,057*	*,070*	*0,74*	*,158*	*,343*
				3HB/AA	*5,78*	*1,19*	*1,33*	*2,76*	*3,27*	*3,40*
HN	19	181	76	Lactate	0,80	8,52	2,35	1,48	2,95	0,89
				Ketones	,024	,021	,014	,034	,014	,256
				3HB/AA	24,0	9,50	14,0	34,0	14,0	6,31
				Lactate	*0,39*	*4,10*	*2,10*	*0,68*	*0,68*	*0,41*
				Ketones	*,046*	*,061*	*,062*	*,043*	*,175*	*,378*
				3HB/AA	*3,18*	*0,85*	*1,00*	*2,58*	*2,72*	*4,25*
JG	19	184	81	Lactate	2,14	2,14	1,90	1,84	1,18	1,32
				Ketones	,024	,034	,024	0,01	,072	,280
				3HB/AA	24,0	34,0	24,0	10,0	72,0	6,40
				Lactate	*1,81*	*1,86*	*2,62*	*1,59*	*0,76*	*1,69*
				Ketones	*,039*	*,054*	*,057*	*,045*	*,106*	*,113*
				3HB/AA	*1,60*	*1,57*	*1,38*	*2,21*	*2,03*	*3,04*
SD	19	170	66	Lactate	0,52	2,10	0,21	0,21	0,10	0,80
				Ketones	,087	,068	,065	,079	,144	,196
				3HB/AA	0,10	1,19	1,95	1,19	1,00	0,96
				Lactate	*0*	*4,11*	*0,48*	*0,24*	*0,17*	*0,72*
				Ketones	*,078*	*,138*	*,049*	*,060*	*,086*	*,186*
				3HB/AA	*0,44*	*0,28*	*0,81*	*0,82*	*2,18*	*2,44*
DP	20	176	65	Lactate	0,41	2,49	0,49	0,52	1,65	0,93
				Ketones	,118		,071	,124	,260	,449
				3HB/AA	2,37		2,55	1,64	2,17	2,48
				Lactate	*3,37*	*2,12*	*0,67*	*0,82*	*0,67*	*0,94*
				Ketones	*,107*	*,065*	*,051*	*,070*	*,145*	*,467*
				3HB/AA	*0,59*	*1,41*	*0,89*	*(70,0)*	*1,26*	*2,65*

CONTROL
ATHLETES

	Age yrs	Height cm	Mass kg		07h30	08h00	09h30	10h30	13h00	17h00
				Lactate	0,91	0,26	0,20	0,42	0,67	0,26
TM	26	186	76	Ketones	,054	,055	,031	,083	,056	,022
				3HB/AA	1,35	2,24	2,10	2,61	5,22	21,0
				Lactate	0,89	0,59	0,48	0,53	0,53	1,04
SO	20	182	74	Ketones	,051	,007	,015	,015	,022	,139
				3HB/AA	0,46	0,40	0,88	14,0	1,75	2,56
				Lactate	0,74	0,73	0,50	1,16	0,71	0,62
MMc	19	177	61	Ketones	,045	,003	,025	,007	,011	,037
				3HB/AA	0,55	2,00	0,39	2,50	10,0	5,17
				Lactate	1,40	1,06	0,77	0,98	0,46	0,58
PS	19	172	66	Ketones	,038	,096	,044	,075	,167	,129
				3HB/AA	2,17	3,80	4,50	2,75	5,96	3,78
				Lactate	1,15	0,58	0,93	0,89	1,61	0,58
NB	19	178	74	Ketones	,109	0,05	,022	,063	,098	,164
				3HB/AA	2,30	1,08	0,83	2,50	3,08	3,32
				Lactate						
FH	19	176	83	Ketones						
				3HB/AA						
				Lactate	1,04	1,73	0,93	1,09	1,38	0,45
DY*	19	180	98	Ketones	,085	,061	,006	,004	,041	,026
				3HB/AA	0,60	0,53	5,00	3,00	0,46	2,71
				Lactate	0,96	0,76	0,49	0,99	0,24	0,89
MB*	19	179	79	Ketones	,067	,027	,006	,043	,040	,156
				3HB/AA	0,02	8,00	5,00	0,23	0,90	1,29
				Lactate	0,43	0,47	0,78	0,58	0,93	1,07
CH*	19	192	101	Ketones	,031	,009	,039	,036	,044	,279
				3HB/AA	0,11	8,00	0,03	0,03	0,91	5,98

* Western Province swimmers

Swim without breathholding
Swim with breathholding

↓SWIM

		07h30	08h15	09h30	10h30	13h00	17h00
TM	Lactate	0,91	3,01		0,25	0,33	0,19
	Ketones	,054	,035		,032	,103	,070
	3HB/AA	1,35	1,19		0,78	2,40	1,59
	Lactate	*0,75*	*2,66*	*0,27*	*0,27*	*0,66*	*0,42*
	Ketones	*,014*	*,010*	*,010*	*,021*	*,085*	*,057*
	3HB/AA	*14,0*	*10,0*	*10,0*	*2,00*	*3,25*	*(57,0)*
SO	Lactate	0,89	4,00	7,80	1,34	1,98	1,36
	Ketones	,031	,042	,037	,029	,057	,076
	3HB/AA	0,82	2,81	3,11	1,42	2,80	2,14
	Lactate	*2,58*	*2,74*	*1,01*	*2,04*	*1,13*	*4,29*
	Ketones	*,051*	*,057*	*,036*	*,078*	*,186*	*,445*
	3HB/AA	*0,46*	*0,39*	*0,24*	*0,90*	*2,15*	*2,03*
MMc	Lactate	0,74	4,80	1,15	1,34	0,74	1,20
	Ketones	,032	,069	,063	,117	,162	,342
	3HB/AA	4,33	3,06	2,50	2,44	2,60	4,43
	Lactate	*0,74*	*6,50*	*0,89*	*0,74*	*1,03*	*0,80*
	Ketones	*,045*	*,041*	*,048*	*,018*	*,107*	*,193*
	3HB/AA	*0,55*	*0,41*	*0,66*	*0,05*	*1,61*	*2,01*
PS	Lactate	1,38	3,55	1,53	1,51	1,61	1,07
	Ketones	,018	,029	,032	,056	,202	,323
	3HB/AA	17,0	4,80	32,0	3,31	13,4	13,6
	Lactate	*1,15*	*6,67*	*1,26*	*0,85*	*1,28*	*1,38*
	Ketones	*,038*	*,073*	*,047*	*0,07*	*,133*	*,196*
	3HB/AA	*2,17*	*1,21*	*4,22*	*1,00*	*3,03*	*5,53*
NB	Lactate	1,15	3,03	0,60	1,98	0,91	1,22
	Ketones	,109	,062	,031	,044	,075	,521
	3HB/AA	2,30	1,14	3,43	0,91	1,34	3,17
	Lactate	*2,37*	*2,89*	*2,33*	*0,93*	*0,64*	*1,72*
	Ketones	*,093*	*,043*	*,081*	*,067*	*,270*	*,186*
	3HB/AA	*2,87*	*1,53*	*2,86*	*3,78*	*2,91*	*10,6*

↓SWIM

		07h30	08h15	09h30	10h30	13h00	17h00
FH	Lactate	0,41	0,70	0,31	0,64	0,58	0,83
	Ketones	,043	,084	,051	,075	0,06	,092
	3HB/AA	0,79	0,53	0,89	1,27	4,00	1,09
	Lactate	*0,79*	*1,39*	*0,84*	*0,77*	*0,22*	*1,44*
	Ketones	*,077*	*,062*	*,087*	*,144*	*,111*	*,211*
	3HB/AA	*0,45*	*0,55*	*0,85*	*0,95*	*0,85*	*1,09*
DY*	Lactate	1,04	0,24	0,48	0,48	0,48	0,96
	Ketones	,085	,066	,063	,198	,132	,302
	3HB/AA	0,60	0,65	1,42	14,2	1,49	1,85
	Lactate	*0,82*		*0,72*	*1,55*	*0,68*	*0,82*
	Ketones	*,052*		*,042*	*,045*	*,066*	*,330*
	3HB/AA	*0,02*		*42,0*	*2,00*	*(66,0)*	*10,0*
MB*	Lactate	0,96	0,48	0,72	0,24	0,96	0,96
	Ketones	,066	,079	,119	,145	,039	,214
	3HB/AA	0,01	0,01	0,28	1,19	2,00	2,24
	Lactate	*0,93*		*1,03*	*0,93*	*0,78*	*0,89*
	Ketones	*,092*		*,033*	*,143*	*,106*	*,314*
	3HB/AA	*0,01*		*1,20*	*0,60*	*2,08*	*4,23*
CH*	Lactate	0,93	0,66	0,47	0,23	0,72	0,54
	Ketones	,031	,083	0,05	,044	,056	,227
	3HB/AA	0,11	0,73	1,17	1,59	2,24	1,18
	Lactate	*1,64*	*0,84*	*0,89*	*0,94*	*1,11*	*0,50*
	Ketones	*,086*	*,106*	*,036*	*,059*	*,024*	*,080*
	3HB/AA	*0,59*	*0,77*	*35,0*	*0,48*	*0,85*	*1,00*

* Western Province Swimmers

CONTROL

NON-ATHLETES

	07h30	08h00	08h30	10h30	13h00	17h00
Lactate	1,08	1,00	0,57	1,17	0,69	0,69
	0,21	*0,19*	*0,08*	*0,24*	*0,19*	*0,18*
Ketones	,044	,047	,036	,050	,078	,209
	,007	*,014*	*,009*	*,010*	*,016*	*,044*
3HB/AA	1,92	2,95	7,37	7,33	3,14	2,84
	0,83	*1,74*	*4,13*	*5,00*	*1,58*	*0,54*

RUNNERS

Lactate	1,02	0,64	0,58	0,80	0,80	0,62
	0,12	*0,13*	*0,13*	*0,14*	*0,21*	*0,12*
Ketones	,059	,042	,027	,049	,071	,098
	,013	*,017*	*,005*	*,016*	*,028*	*,029*
3HB/AA	1,31	1,90	1,74	4,87	5,20	7,17
	0,36	*0,58*	*0,75*	*2,28*	*1,41*	*3,48*

SWIMMERS

Lactate	0,81	0,99	0,73	0,89	0,85	0,80
	0,19	*0,38*	*0,13*	*0,16*	*0,33*	*0,18*
Ketones	,061	,032	,017	,028	,042	,154
	,015	*,015*	*,011*	*,012*	*,001*	*,073*
3HB/AA	0,24	5,51	3,34	1,09	0,76	3,15
	0,18	*2,49*	*1,66*	*0,96*	*0,15*	*1,44*

Mean = Roman type

Standard Error of Mean = italics

SWIMMING WITHOUT BREATHHOLDING

NON-ATHLETES

	07h30	08h15	09h30	10h30	13h00	17h00
Lactate	1,16	3,21	1,12	1,21	1,21	0,96
	0,18	*0,71*	*0,23*	*0,23*	*0,29*	*0,14*
Ketones	*,050*	,046	,038	,072	,115	,266
	,010	*,008*	*,008*	*,019*	*,029*	*,036*
3HB/AA	9,15	12,2	7,65	8,78	16,86	3,68
	3,34	*4,69*	*2,61*	*3,90*	*9,11*	*0,82*

RUNNERS

Lactate	0,91	3,18	2,28	1,18	0,95	0,98
	0,14	*0,57*	*1,40*	*0,26*	*0,24*	*0,17*
Ketones	,048	,053	,043	,059	*,110*	,237
	,013	*,009*	*,006*	*,014*	*,024*	*,076*
3HB/AA	4,43	2,26	8,39	1,69	4,42	4,34
	2,57	*0,65*	*5,92*	*0,40*	*1,83*	*1,92*

SWIMMERS

Lactate	0,98	0,46	0,56	0,32	0,72	0,82
	0,03	*0,12*	*0,08*	*0,08*	*0,14*	*0,14*
Ketones	0,61	,076	,077	,129	,076	,248
	,016	*,005*	*,021*	*,045*	*,029*	*,027*
3HB/AA	0,24	0,46	0,96	5,66	1,91	1,76
	0,18	*0,23*	*0,35*	*4,27*	*0,22*	*0,31*

Mean = Roman type

Standard Error of Mean = italics

SWIMMING WITH BREATHHOLDING

NON-ATHLETES

	07h30	08h15	09h30	10h30	13h00	17h00
Lactate	1,45	3,43	1,10	1,04	0,60	0,92
	0,39	*0,38*	*0,22*	*0,18*	*0,12*	*0,14*
Ketones	0,72	,064	,053	,061	,146	,334
	,022	*,011*	*,007*	*,016*	*,020*	*,047*
3HB/AA	1,72	4,01	2,02	4,06	4,66	3,15
	0,06	*3,15*	*0,95*	*1,94*	*2,44*	*0,35*

RUNNERS

Lactate	1,40	3,81	1,10	0,93	0,83	1,68
	0,35	*0,91*	*0,28*	*0,24*	*0,16*	*0,56*
Ketones	,053	,048	,052	,066	,149	,215
	,012	*,009*	*,012*	*,019*	*,028*	*,052*
3HB/AA	3,42	2,35	3,14	1,45	2,30	4,25
	2,16	*1,54*	*1,51*	*0,53*	*0,38*	*1,76*

SWIMMERS

Lactate	1,13	0,84	0,88	1,14	0,86	0,74
	0,26		*0,09*	*0,21*	*0,13*	*0,12*
Ketones	,077	,106	,037	,082	,065	,241
	,012		*,026*	*,031*	*,024*	*,080*
3HB/AA	0,21	0,77	26,1	1,03	1,47	5,08
	0,19		*12,6*	*0,49*	*0,61*	*2,63*

Mean = Roman type

Standard error of Mean = italics

VARYING WORK LOADS

CONTROL

NON-ATHLETES

	Age yrs	Height cm	Mass kg		07h30	08h00	09h30	13h00	17h00
				Lactate	2,93	2,36	1,72	2,48	1,20
RW	19	172	67	Ketones	,021	,039	,025	,030	,212
				3HB/AA	2,00	2,00	3,17	2,75	3,33
				Lactate	1,42	0,71	0,54	1,14	0,89
RV	19	184	66	Ketones	,095	,045	,035	,045	,082
				3HB/AA	3,13	1,14	1,69	2,21	0,55
				Lactate	1,07	0,85	0,60	0,68	1,28
AF	19	172	60	Ketones	,076	,023	,032	,018	,091
				3HB/AA	1,38	2,83	1,46	2,00	2,78
				Lactate	0,89	0,95	0,76	0,74	0,14
HN	19	181	76	Ketones	,025	,076	,073	,077	,061
				3HB/AA	2,13	4,43	1,92	1,83	3,69
				Lactate	2,09	0,41	0,41	0,71	0,35
KS	19	177	70	Ketones	,079	,040	,043	,163	,162
				3HB/AA	0,41	4,71	1,26	2,33	5,23
				Lactate	0,71	0,70	0,62	0,66	0,86
ED	19	178	69	Ketones	,022	,031	,032	,096	,154
				3HB/AA	2,14	1,58	1,13	1,67	3,16
				Lactate	0,45	0,93	0,85	1,01	0,80
BM	19	175	74	Ketones	,039	,100	,047	,130	,226
				3HB/AA	0,07	0,52	1,24	1,46	1,28
				$\bar{x}$	1,37	0,99	0,79	1,06	0,79
Lactate				SD	0,87	0,63	0,44	0,65	0,42
				SEM	0,33	0,24	0,16	0,25	0,16
				$\bar{x}$	,051	,051	,041	,080	,141
Ketones				SD	,031	,027	,016	,054	,065
				SEM	,012	,010	,006	,020	,025
				$\bar{x}$	1,61	2,46	1,70	2,04	2,86
3HB/AA				SD	1,07	1,61	0,71	0,44	1,55
				SEM	0,40	0,61	0,27	0,16	0,59

		↓ 75W for 15 minutes					HEART RATE	
		07h30	08h00	09h30	13h00	17h00	Before	After
RW	Lactate	2,93	1,78	3,59	1,75	2,17	88	147
	Ketones	,051	,033	,014	,020	,139		
	3HB/AA	1,55	3,71	2,50	2,33	1,84		
RV	Lactate	0,42	1,78	0,54	1,57	3,28	80	135
	Ketones	,057	,030	,029	,055	,314		
	3HB/AA	1,71	2,33	2,63	3,58	2,24		
AF	Lactate	1,87	1,11	1,09	1,53	0,74	60	100
	Ketones	,030	,026	,032	,036	,024		
	3HB/AA	0,87	1,17	1,46	1,40	1,00		
HN	Lactate	0,88	0,49	0,58	1,32	1,20	75	110
	Ketones	,017	,014	,014	,009	,216		
	3HB/AA	1,42	7,00	1,75	1,50	2,20		
KS	Lactate	1,59	0,32	0,96	0,67	0,77	64	109
	Ketones	,062	,067	,074	,142	,431		
	3HB/AA	1,38	1,48	1,85	2,84	24,0		
ED	Lactate	0,51	0,98	1,06	0,51	0,59	76	120
	Ketones	,018	,007	,014	,022	,308		
	3HB/AA	2,00	0,14	2,50	3,40	3,53		
BM	Lactate	0,56	1,71	0,78	1,79	1,57	64	100
	Ketones	,032	,021	,013	,018	,061		
	3HB/AA	0,42	1,61	13,0	0,13	0,66		
Lactate	$\bar{x}$	1,25	1,17	1,23	1,31	1,47	72	117
	SD	0,93	0,61	1,06	0,51	0,97	10	18
	SEM	0,35	0,23	0,40	0,19	0,37	4	7
Ketones	$\bar{x}$	,038	,028	,027	,043	,213		
	SD	,018	,019	,022	,046	,148		
	SEM	,007	,007	,008	,017	,056		
3HB/AA	$\bar{x}$	1,34	2,49	3,67	2,17	5,61		
	SD	0,53	2,27	4,14	1,24	9,07		
	SEM	0,20	0,86	1,56	0,47	3,70		

		↓ 100W for 15 minutes					HEART RATE	
		07h30	08h00	09h30	13h00	17h00	Before	After
RW	Lactate	2,43	4,17	1,07	1,07	0,66	88	172
	Ketones	,021	,028	,021	,012	,192		
	3HB/AA	2,00	1,54	9,50	0,08	2,37		
RV	Lactate	1,55	2,70	0,93	1,07	0,21	88	166
	Ketones	,095	,044	,032	,020	,453		
	3HB/AA	3,13	1,20	1,46	1,00	2,33		
AF	Lactate	1,14	0,73	0,79	0,58	0,42	67	124
	Ketones	,076	,051	,045	,362	,040		
	3HB/AA	1,37	2,64	1,65	2,77	1,11		
HN	Lactate	0,60	0,91	0,35	0,66	0,97	70	126
	Ketones	,017	,007	,014	,033	,038		
	3HB/AA	2,12	0,50	3,50	3,00	5,43		
KS	Lactate	0,33	0,39	0,93	1,18	0,89	80	114
	Ketones	,079	,046	,112	,182	,297		
	3HB/AA	2,43	22,0	1,15	1,56	1,70		
ED	Lactate	0,58	1,63	0,56	0,72	0,39	80	138
	Ketones	,022	,031	,019	,098	,392		
	3HB/AA	2,14	0,72	0,05	2,27	2,92		
BM	Lactate	1,53	2,56	2,43	1,19	0,89	69	126
	Ketones	,024	,021	,021	,045	,093		
	3HB/AA	1,60	0,54	0,81	4,09	2,07		
Lactate	$\bar{x}$	1,17	1,87	1,01	0,92	0,63	77	138
	SD	0,73	1,35	0,67	0,26	0,30	9	22
	SEM	0,28	0,51	0,25	0,10	0,11	3	8
Ketones	$\bar{x}$	,048	,033	,038	,107	,215		
	SD	,034	,016	,034	,127	,169		
	SEM	,013	,006	,013	,048	,064		
3HB/AA	$\bar{x}$	2,11	4,16	2,59	2,11	2,56		
	SD	0,57	7,90	3,23	1,34	1,39		
	SEM	0,22	2,99	1,22	0,51	0,52		

		↓125W for 15 minutes					HEART RATE		
		07h30	08h00	09h30	13h00	17h00	Before	After	
RW	Lactate	5,98	5,16	1,88	1,92	2,87	84	190	Maximal
	Ketones	,077	,047		,039	,054			
	3HB/AA	1,08	2,92		1,44	2,85			
RV	Lactate	4,37	6,31	0,84	1,28	3,57	105	200	Maximal
	Ketones	,207	,082	,138	,084	,218			
	3HB/AA	1,22	3,56	0,31	1,63	2,25			
AF	Lactate	2,67	1,68	1,44	1,23	0,53	71	138	
	Ketones	,036	,063	,042	,053	,073			
	3HB/AA	2,27	2,70	3,20	2,78	2,04			
HN	Lactate	0,70	2,50	0,31	1,94	0,39	78	154	
	Ketones	,017	,017	,028	,012	,348			
	3HB/AA	1,50	2,12	4,00	1,09	3,19			
KS	Lactate	1,01	1,57	0,31	0,70	0,41	66	132	
	Ketones	,039	,033	,027	,060	,335			
	3HB/AA	3,87	15,5	26,0	0,66	2,85			
ED	Lactate	0,56	2,39	0,52	0,49	0,74	92	167	
	Ketones	,037	,043	,033	,081	,383			
	3HB/AA	2,36	2,58	1,36	2,86	3,73			
BM	Lactate	1,03	2,66	2,39	5,77	1,15	59	142	
	Ketones	,018	,032	,011	,124	,153			
	3HB/AA	0,28	32,0	11,0	1,02	0,77			
Lactate	$\bar{x}$	2,33	3,18	1,10	1,90	1,38	79	160	
	SD	2,11	1,82	0,82	1,79	1,30	16	26	
	SEM	0,80	0,69	0,31	0,68	0,49	6	10	
Ketones	$\bar{x}$	,062	,045	,047	,065	,223	73*	147*	*RW and RV excluded
	SD	,067	,022	,046	,036	,135	13	14	
	SEM	,025	,008	,019	,014	,051	6	6	
3HB/AA	$\bar{x}$	1,80	8,77	7,65	1,64	2,53			
	SD	1,16	1,30	9,74	0,86	0,96			
	SEM	0,44	4,27	3,98	0,33	0,36			

		↓ 150W for 15 minutes					HEART RATE	
		07h30	08h00	09h30	13h00	17h00	Before	After
RW	Lactate							
	Ketones							
	3HB/AA							
HN[1]	Lactate	0,91	2,70	0,39	0,56	0,76		
	Ketones	,031	,026	,031	,195	,086	71	163
	3HB/AA	1,55	1,73	3,10	3,15	1,75		
AF	Lactate	0,62	2,58	0,85	0,25	0,56		
	Ketones	,086	,066	,062	,067	,048	60	155
	3HB/AA	3,30	2,14	2,26	3,47	1,40		
HN[2]	Lactate	0,91	2,70	0,52	0,43	0,19		
	Ketones	,050	,045	,043	,055	,061	70	167
	3HB/AA	1,43	4,50	3,58	3,67	1,65		
KS	Lactate	0,59	2,26	0,69	1,43	0,63		
	Ketones	,015	,026	,022	,021	,030	84	148
	3HB/AA	0,87	26,0	1,75	1,33	2,33		
ED	Lactate	0,40	3,76	1,08	1,43	0,48		
	Ketones	,047	,026	,045	,171	,262	84	182
	3HB/AA	5,71	26,0	5,43	0,34	3,09		
BM	Lactate	1,88	2,02	1,38	0,66	0,52		
	Ketones	,018	,026	,008	,084	,196	64	151
	3HB/AA	18,0	0,47	0,12	(84,0)	1,57		
Lactate	$\bar{x}$	0,89	2,67	0,82	0,79	0,52	72	161
	SD	0,53	0,60	0,37	0,51	0,19	10	12
	SEM	0,21	0,24	0,15	0,21	0,08	4	5
Ketones	$\bar{x}$	,041	,036	,035	,099	,114		
	SD	,026	,017	,019	,069	,093		
	SEM	,011	,007	,008	,028	,038		
3HB/AA	$\bar{x}$	5,14	10,1	2,71	2,39	1,97		
	SD	6,54	12,4	1,80	1,48	0,64		
	SEM	2,67	5,04	0,73	0,66	0,26		

		↓MAXIMUM for 15 minutes						HEART RATE	
		07h30	08h00	09h30	13h00	17h00	Load W	Before	After
RW	Lactate	5,98	5,16	1,88	1,92	2,87	125	84	190
	Ketones	,077	,047		,039	,054			
	3HB/AA	1,08	2,92		1,44	2,86			
RV	Lactate	4,37	6,31	0,84	1,28	3,57	125	105	200
	Ketones	,207	,082	,138	,084	,218			
	3HB/AA	1,22	3,56	0,31	1,63	2,25			
AF	Lactate	0,37	6,19	0,39	0,82	0,19	170	65	190
	Ketones	,256	,128	,231	,300	,704			
	3HB/AA	3,00	2,37	2,67	3,11	2,91			
HN	Lactate	0,52	3,96	0,43	0,47	0,56	175	80	188
	Ketones	,071	,036	,129	,138	,162			
	3HB/AA	2,63	1,71	2,30	2,46	2,13			
KS	Lactate	0,54	7,20	1,32	1,38	1,01	210	74	184
	Ketones	,033	,043	,024	,038	,245			
	3HB/AA	0,57	2,07	0,71	0,81	2,55			
ED	Lactate	1,48	7,26	1,71	0,78	0,56	170	96	200
	Ketones	,047	,047	,031	,148	,284			
	3HB/AA	1,61	2,36	3,43	2,22	3,73			
BM	Lactate	1,32	7,36	1,30	0,62	0,41	225	64	188
	Ketones	,037	,060	,039	,090	,042			
	3HB/AA	2,50	1,93	2,10	2,64	2,34			
Lactate	$\bar{x}$	2,08	6,20	1,12	1,04	1,31	171	81	191
	SD	2,20	1,26	0,59	0,51	1,34	38	15	6
	SEM	0,83	0,48	0,22	0,19	0,51	14	6	2
Ketones	$\bar{x}$	,104	,063	,098	,120	,244			
	SD	,090	,032	,082	,090	,223			
	SEM	,034	,012	,033	,034	,084			
3HB/AA	$\bar{x}$	1,80	2,42	1,92	2,04	2,68			
	SD	0,91	0,64	1,19	0,79	0,55			
	SEM	0,35	0,24	0,49	0,30	0,21			

CONTROL

ATHLETES

	Age yrs	Height cm	Mass kg		07h30	08h00	09h30	13h00	17h00
				Lactate	1,55	0,35	0,45	1,20	1,15
KO	18	188	86	Ketones	,028	,013	,026	,079	,142
				3HB/AA	1,33	12,0	0,63	2,29	1,68
				Lactate	1,14	0,64	1,11	1,15	1,40
KP	19	185	75	Ketones	,035	,037	,080	,066	,131
				3HB/AA	2,18	2,36	1,29	1,54	2,45
				Lactate	0,32	0,62	0,14	0,27	0,70
PB	19	177	75	Ketones	,029	,048	,022	,005	,019
				3HB/AA	2,63	0,65	21,0	0,25	2,80
				Lactate	0,54	0,64	0,33	0,33	0,85
RS	23	177	75	Ketones	,025	,017	,019	,086	,040
				3HB/AA	1,06	0,70	0,58	4,73	3,44
				Lactate	0,37	0,33	0,52	0,23	0,31
SH	20	176	71	Ketones	,105	,047	,028	,067	,069
				3HB/AA	2,50	2,62	13,0	1,68	1,56
				Lactate	0,62	0,27	0,74	0,31	0,37
MM	19	177	61	Ketones	,056	,063	,056	,050	,131
				3HB/AA	3,31	4,73	3,31	6,14	2,97
				Lactate					
KR	18	183	69	Ketones					
				3HB/AA					
				$\bar{x}$	0,76	0,48	0,55	0,58	0,80
	Lactate			SD	0,49	0,18	0,34	0,46	0,43
				SEM	0,20	0,07	0,14	0,19	0,18
				$\bar{x}$	,046	,038	,038	,059	,089
	Ketones			SD	,031	,019	,024	,029	,053
				SEM	,013	,008	,010	,012	,022
				$\bar{x}$	2,17	3,84	6,64	2,77	2,48
	3HB/AA			SD	0,84	4,27	8,48	2,21	0,74
				SEM	0,34	1,74	3,46	0,90	0,30

		↓ 75W for 15 minutes					HEART RATE	
		07h30	08h00	L9h30	13h00	17h00	Before	After
	Lactate	1,55	0,70	0,35	0,31	0,49		
KO	Ketones	,009	,022	,018	,040	,014	76	108
	3HB/AA	4,68	2,97	3,05	14,25	4,04		
	Lactate	1,14	0,28	1,68	0,53	0,65		
KP	Ketones	,085	,081	,087	,106	,179	60	84
	3HB/AA	1,29	2,11	2,48	2,53	3,16		
	Lactate	0,32	0,32	0,75	0,38	0,32		
PB	Ketones	,025	,040	,036	,070	,021	88	100
	3HB/AA	1,27	2,64	2,00	4,38	0,50		
	Lactate	0,54	0,45	1,09	0,56	0,45		
RS	Ketones	,014	,015	,020	,043	,042	56	94
	3HB/AA	0,16	0,15	1,00	1,26	1,00		
	Lactate	0,85	0,47	0,21	0,80	1,05		
SH	Ketones	,115	,093	,040	,119	,071	51	100
	3HB/AA	6,05	0,45	40,0	0,72	0,82		
	Lactate	0,62	0,35	0,45	0,68	0,91		
MM	Ketones	,052	,021	,019	,005	,175	75	110
	3HB/AA	3,71	21,0	3,80	2,50	3,30		
	Lactate	0,64	0,49	0,60	0,54	0,49		
KR	Ketones	,142	,127	,085	,061	,131	72	112
	3HB/AA	1,25	1,75	1,25	1,86	1,00		
	$\bar{x}$	0,81	0,44	0,73	0,54	0,62	68	101
Lactate	SD	0,42	0,14	0,51	0,17	0,27	13	10
	SEM	0,16	0,05	0,19	0,06	0,10	5	4
	$\bar{x}$	,063	,057	,044	,063	,090		
Ketones	SD	,052	,043	,030	,039	,071		
	SEM	,020	,016	,011	,015	,027		
	$\bar{x}$	2,63	4,44	7,65	3,93	1,97		
3HB/AA	SD	2,19	7,38	14,3	4,70	1,46		
	SEM	0,83	2,79	5,40	1,78	0,55		

		↓ 100W for 15 minutes					HEART RATE	
	Lactate	07h30	08h00	09h30	13h00	17h00	Before	After
	Lactate	1,07	0,64	0,54	0,56	1,07		
KO	Ketones	,015	,023	,028	,014	,194	78	122
	3HB/AA	0,15	0,44	1,54	2,50	1,34		
	Lactate	0,87	0,58	0,39	0,49	0,70		
KP	Ketones	,009	,021	,015	,011	,163	72	96
	3HB/AA	0,11	2,50	0,67	2,67	1,26		
	Lactate	0,35	0,27	0,23	0,12	0,43		
PB	Ketones	,044	,027	,039	,033	,041	99	117
	3HB/AA	0,69	2,86	2,25	4,50	0,46		
	Lactate	0,48	0,65		0,52	0,25		
RS	Ketones	,036	,014		,131	,042	52	102
	3HB/AA	1,00	1,80		2,54	1,47		
	Lactate	0,39	0,49	0,49	0,33	0,47		
SH	Ketones	,075	,061	,074	,167	,153	52	108
	3HB/AA	2,50	2,35	3,52	0,51	3,82		
	Lactate	0,39	0,39	0,56	0,47	0,72		
MM	Ketones	,043	,021	,017	,021	,090	58	115
	3HB/AA	3,31	1,50	2,43	2,33	3,91		
	Lactate	0,29	0,70	0,45	0,49	0,80		
KR	Ketones	,069	,050	,024	,110	,314	87	124
	3HB/AA	2,00	2,57	24,0	2,55	0,75		
	$\bar{x}$	0,55	0,53	0,44	0,43	0,63	71	112
Lactate	SD	0,30	0,16	0,12	0,15	0,27	18	10
	SEM	0,11	0,06	0,05	0,06	0,10	7	4
	$\bar{x}$	,042	,031	,033	,070	,142		
Ketones	SD	,025	,017	,022	,065	,096		
	SEM	,009	,007	,009	,024	,036		
	$\bar{x}$	1,39	2,00	5,74	2,51	1,86		
3HB/AA	SD	1,23	0,83	9,00	1,16	1,42		
	SEM	0,47	0,31	3,67	0,44	0,53		

		↓ 125W for 15 minutes					HEART RATE	
		07h30	08h00	09h30	13h00	17h00	Before	After
KO	Lactate	1,59	0,93	0,97	0,97	0,93	70	124
	Ketones	,028	,040	,009	,007	,085		
	3HB/AA	1,33	0,54	3,50	0,14	0,49		
KP	Lactate	0,47	1,32	1,07	0,70	0,91	56	108
	Ketones	,035	,026	,041	,018	,026		
	3HB/AA	2,18	4,20	1,41	2,00	0,86		
PB	Lactate							
	Ketones							
	3HB/AA							
RS	Lactate	2,69	1,15	0,30	0,24	0,30	53	120
	Ketones	,025	,021	,034	,150	,351		
	3HB/AA	2,57	20,0	2,09	2,85	2,90		
SH	Lactate	0,54	0,58	0,45	0,14	0,27	52	122
	Ketones	,166	,101	,100	,200	,135		
	3HB/AA	1,48	(100)	1,09	5,06	3,03		
MM	Lactate	0,52	0,89	0,85	0,43	0,74	62	129
	Ketones	,197	,085	,069	,130	,256		
	3HB/AA	2,81	2,50	1,92	3,42	3,32		
KR	Lactate	0,21	0,43	0,62	0,35	0,76	112	138
	Ketones	,026	,037	,061	,070	,165		
	3HB/AA	2,25	1,64	1,65	2,04	2,43		
Lactate	$\bar{x}$	1,00	0,88	0,71	0,47	0,65	67	123
	SD	0,95	0,34	0,30	0,31	0,29	23	10
	SEM	0,39	0,14	0,12	0,13	0,12	9	4
Ketones	$\bar{x}$	,079	,052	,052	,096	,169		
	SD	,080	,033	,032	,077	,118		
	SEM	,033	,014	,013	,031	,048		
3HB/AA	$\bar{x}$	2,10	5,78	1,94	2,59	2,17		
	SD	0,59	8,06	0,84	1,64	1,20		
	SEM	0,24	3,61	0,34	0,67	0,49		

		↓ 150W for 15 minutes					HEART RATE	
		07h30	08h00	09h30	13h00	17h00	Before	After
	Lactate	0,62	1,65	0,75	1,22	0,58		
KO	Ketones	,036	,058	,023	,023	,018	64	144
	3HB/AA	2,00	2,41	1,09	2,83	3,50		
	Lactate	0,61	0,40	1,14	1,14	0,69		
KP	Ketones	,069	,060	,057	,203	,112	72	116
	3HB/AA	3,93	4,00	3,75	2,03	1,60		
	Lactate	0,89	0,39	0,33	0,23	0,87		
PB	Ketones	,029	,030	,027	,014	,029	90	136
	3HB/AA	2,62	6,50	1,70	6,00	1,90		
	Lactate	0,29	1,92	0,60	0,12	0,27		
RS	Ketones	,051	,046	,037	,083	,363	63	134
	3HB/AA	5,38	6,67	5,17	2,95	3,22		
	Lactate	0,29	0,78	0,25	0,27	0,23		
SH	Ketones	,193	,076	,132	,203	,058	40	128
	3HB/AA	1,95	1,62	18,8	1,74	0,98		
	Lactate	0,37	0,85	0,66	0,45	0,60		
MM	Ketones	,036	,026	,024	,038	,024	60	130
	3HB/AA	3,27	1,53	24,0	4,75	2,18		
	Lactate	0,27	0,46	0,23	0,50	0,25		
KR	Ketones	,054	,044	,029	,043	,109	88	150
	3HB/AA	2,18	4,50	4,80	2,58	2,20		
	$\bar{x}$	0,48	0,92	0,57	0,56	0,50	68	134
Lactate	SD	0,24	0,62	0,33	0,44	0,25	17	11
	SEM	0,09	0,24	0,12	0,17	0,09	7	4
	$\bar{x}$	,067	,049	,047	,087	,102		
Ketones	SD	,057	,018	,039	,082	,122		
	SEM	,022	,007	,015	,031	,046		
	$\bar{x}$	3,05	3,89	8,47	3,27	2,23		
3HB/AA	SD	1,26	2,15	9,08	1,54	0,88		
	SEM	0,48	0,81	3,43	0,58	0,33		

		↓ MAXIMUM for 15 minutes						HEART RATE		
		07h30	08h00	09h30	13h00	17h00	Load W	Before	After	
PB	Lactate	1,84	1,22	1,20	0,45	0,47	230	96	161	Not maximal
	Ketones	,024	,051	,020	,036	,049				
	3HB/AA	0,71	1,55	19,0	0,89	2,77				
KP	Lactate	0,66	6,37	2,21	1,46	0,85	250	92	192	
	Ketones	,022	,060	,030	,168	,099				
	3HB/AA	0,83	5,00	1,72	1,40	1,36				
PB	Lactate	0,57	5,43	0,73	0,48	1,04	250	88	184	
	Ketones	,093	,077	,042	,120	,114				
	3HB/AA	2,58	1,26	3,67	4,45	1,65				
RS	Lactate	0,82	6,72	0,62		1,48	220	58	172	
	Ketones	,021	,063	,055		,095				
	3HB/AA	0,50	2,15	1,50		1,50				
SH	Lactate	0,37	7,28	0,58	0,60	0,45	280	50	180	
	Ketones	,061	,066	,037	,074	,013				
	3HB/AA	0,02	0,62	0,92	0,83	6,15				
MM	Lactate	0,58	5,40	0,56	0,97	0,29	225	74	182	
	Ketones	,050	,074	,040	,033	,050				
	3HB/AA	3,33	3,89	5,71	1,94	4,17				
KR	Lactate									
	Ketones									
	3HB/AA									
Lactate	$\bar{x}$	0,60	6,24	0,94	0,88	0,82	245	72	182	
	SD	0,16	0,82	0,71	0,44	0,48	24	18	7	
	SEM	0,07	0,37	0,32	0,22	0,21	11	8	3	
Ketones	$\bar{x}$	,049	,068	,041	,099	,074				
	SD	,030	,007	,009	,058	,042				
	SEM	,013	,003	,004	,029	,019				
3HB/AA	$\bar{x}$	1,45	2,58	2,70	2,16	2,97				
	SD	1,43	1,83	1,97	1,60	2,12				
	SEM	0,64	0,82	0,88	0,80	0,95				

DURATION OF EXERCISE

CONTROL

	Age yrs	Height cm	Mass kg		07h30	08h30	09h30	13h00	17h00
HR	19	183	91	Lactate	1,13	0,92	0,88	0,39	0,37
				Ketones	,053	,048	,056	,147	,086
				3HB/AA	7,83	1,18	1,80	2,34	1,96
IK	19	178	58	Lactate	1,13	0,98	2,28	0,36	2,18
				Ketones	,062	,084	,029	,057	,055
				3HB/AA	3,13	0,91	1,90	1,38	0,77
CJ	18	175	55	Lactate	1,29	0,64	0,45	0,78	0,36
				Ketones	,045	,076	,053	,072	,065
				3HB/AA	2,21	1,92	1,41	2,60	1,95
JM	18	169		Lactate	1,07	0,35	0,62	0,95	1,86
				Ketones	,111	,094	,048	,026	,100
				3HB/AA	2,58	3,70	1,82	2,25	2,03
AF	19	172	60	Lactate	1,07	0,85	0,60	0,68	1,28
				Ketones	,076	,023	,032	,018	,091
				3HB/AA	1,38	2,83	1,46	2,00	2,78
ED	19	178	69	Lactate	0,71	0,70	0,62	0,66	0,86
				Ketones	,022	,031	,032	,096	,154
				3HB/AA	2,14	1,58	1,13	1,67	3,16
MT*	20	186		Lactate	0,64	1,71	1,14	0,42	0,21
				Ketones	,088	,048	,056	,133	,063
				3HB/AA	2,38	5,00	3,31	3,75	2,15
KO*	18	188	86	Lactate	1,55	0,35	0,45	1,20	1,15
				Ketones	,028	,013	,026	,079	,142
				3HB/AA	1,33	12,0	0,63	2,29	1,68
PB*	19	177	75	Lactate	0,32	0,62	0,14	0,27	0,70
				Ketones	,029	,048	,022	,005	,019
				3HB/AA	2,63	0,65	21,0	0,25	2,80

* ATHLETES

		↓ ½ hr Exercise					HEART RATE	
		07h30	08h00	09h30	13h00	17h00	Before	After
HR	Lactate	1,13	4,04	0,61	0,28	0,48	64	120
	Ketones	,055	,082	,090	,097	,046		
	3HB/AA	1,12	1,28	5,92	3,85	0,43		
IK	Lactate	1,29	0,96	0,63	0,59	1,15	80	116
	Ketones	,075	,089	,095	,137	,040		
	3HB/AA	1,03	1,47	2,17	1,49	0,90		
CJ	Lactate	1,29	1,48	0,69	0,28	0,26	94	120
	Ketones	,045	,079	,040	,094	,079		
	3HB/AA	2,21	1,08	1,86	5,27	1,55		
JM	Lactate	1,07	1,36	0,54	0,64	0,78	92	148
	Ketones	,111	,156	,100	,219	,062		
	3HB/AA	2,58	3,33	2,57	1,77	0,63		
AF	Lactate	1,49	0,89	0,63	0,65	0,50	77	116
	Ketones	,015	,039	,040	,029	,049		
	3HB/AA	14,0	1,60	2,63	1,90	0,40		
ED	Lactate	1,12	1,12	1,47	1,95	0,51	80	132
	Ketones	,029	,018	,017	,136	,199		
	3HB/AA	1,64	18,0	3,25	3,39	3,06		
MT	Lactate	0,64	0,76	0,80	0,66	0,37	80	120
	Ketones	,088	,090	,078	,127	,116		
	3HB/AA	2,38	1,65	2,00	2,43	1,64		
KO	Lactate	1,04	1,41	0,92	0,42	1,47	66	112
	Ketones	,035	,028	,031	,037	,085		
	3HB/AA	2,18	2,11	2,44	2,36	0,73		
PB	Lactate	0,75	0,51	0,59	0,84	0,88	86	101
	Ketones	,031	,046	,023	,089	,047		
	3HB/AA	2,87	3,18	23,0	3,45	0,40		

		↓ 1 hour Exercise					HEART RATE		
		07h00	08h00	09h30	13h00	17h00	Before	½hr	After
	Lactate	0,93	3,46	0,70	0,78	0,68			
HR	Ketones	,025	,042	,026	,020	,051	72	120	124
	3HB/AA	24,0	2,82	12,0	2,33	2,92			
	Lactate	1,13	1,73	0,95	0,99	1,71			
IK	Ketones	,058	,053	,060	,027	,143	100	120	116
	3HB/AA	2,41	2,53	2,16	4,40	2,92			
	Lactate	2,42	0	0,66	0,17	0,91			
CJ	Ketones	,023	,066	,158	,047	,138	80	120	120
	3HB/AA	3,60	2,88	2,85	1,14	2,21			
	Lactate								
JM	Ketones								
	3HB/AA								
	Lactate	0,53	0,18	0,12	0,24	0,45			
AF	Ketones	,082	,162	,102	,234	,021	65	106	108
	3HB/AA	2,73	2,68	4,10	3,33	9,50			
	Lactate	0,46	0,79	0,25	0,44	0,44			
ED	Ketones	,057	,062	,153	,426	,352	93	134	147
	3HB/AA	4,18	30,0	5,65	3,78	3,96			
	Lactate								
MT	Ketones								
	3HB/AA								
	Lactate	0,49	0,62	0,47	0,18	0,32			
KO	Ketones	,029	,035	,032	,065	,033	72	120	128
	3HB/AA	1,90	3,71	3,00	1,95	15,5			
	Lactate	0,15	0,34	1,26	0,40	0,87			
PB	Ketones	,047	,055	,044	,041	,066	76	105	101
	3HB/AA	46,0	12,0	43,0	41,0	3,18			

		↓ 1½ hr Exercise					HEART RATE		
		06h30	08h00	09h30	13h00	17h00	Before	½hr	After
	Lactate	1,65	1,74	0,77	0,23	0,27			
HR	Ketones	,053	,092	,033	,010	,079	70	132	128
	3HB/AA	7,83	14,0	5,60	0,01	1,32			
	Lactate	1,35	0,77	0,89	0,93	1,88			
IK	Ketones	,062	,185	,297	,184	,034	92	128	124
	3HB/AA	3,13	2,78	2,67	1,45	0,89			
	Lactate	5,28	0,87	0,47	0,30	0,45			
CJ	Ketones	,036	,046	,059	,041	,053		132	124
	3HB/AA	2,00	3,60	3,50	1,73	2,53			
	Lactate	0,37	0,62	0,94	0,49	0,41			
JM	Ketones	,149	,408	,100	,115	,278	80	132	120
	3HB/AA	28,8	1,58	3,00	1,39	1,89			
	Lactate	1,36	0,49	0,35	0,39	1,05			
AF	Ketones	,052	,023	,025	,017	,033	68	108	112
	3HB/AA	1,00	22,0	0,92	1,43	1,06			
	Lactate	0,82	0,90	0,14	0,18	0,24			
ED	Ketones	,041	,084	,160	,235	,431	89	138	152
	3HB/AA	9,25	6,64	2,81	3,52	9,51			
	Lactate	1,79	0,59	0,51	0,45	1,13			
MT	Ketones	,091	,130	,403	,341	,375	80	120	116
	3HB/AA	1,12	2,82	1,80	3,74	2,00			
	Lactate	0,43	1,07	0,70	0,85	0,43			
KO	Ketones	,028	,025	,029	,042	,051	60	110	120
	3HB/AA	1,55	7,33	1,90	2,82	0,89			
	Lactate	0,28	0,37	0,02	0,88	0			
PB	Ketones	,038	,105	,105	,076	,043	78	102	100
	3HB/AA	37,0	8,55	2,75	2,04	2,91			

CONTROL

			07h30	08h00	09h30	13h00	17h00
NON-ATHLETES n=6	Lactate	$\bar{x}$	1,07	0,74	0,91	0,64	1,15
		SD	0,19	0,23	0,69	0,23	0,76
		SEM	0,08	0,09	0,28	0,09	0,31
	Ketones	$\bar{x}$	,062	,059	,042	,069	,092
		SD	,030	,029	,012	,048	,035
		SEM	,012	,012	,005	,019	,014
	3HB/AA	$\bar{x}$	3,21	2,02	1,59	2,04	2,11
		SD	2,33	1,06	0,30	0,45	0,82
		SEM	0,95	0,43	0,12	0,18	0,34
ATHLETES n=3	Lactate	$\bar{x}$	0,84	0,89	0,58	0,63	0,69
		SD	0,64	0,72	0,51	0,50	0,47
		SEM	0,37	0,42	0,30	0,29	0,27
	Ketones	$\bar{x}$	,048	,036	,035	,072	,075
		SD	,034	,020	,018	,064	,062
		SEM	,020	,012	,011	,037	,036
	3HB/AA	$\bar{x}$	2,11	5,88	8,31	2,10	2,21
		SD	0,69	5,73	11,07	1,76	0,56
		SEM	0,40	3,31	6,39	1,01	0,32

			Resting	Before exercise
NON-ATHLETES	Heart rate	$\bar{x}$	81	
		SD	11	
		SEM	3	
ATHLETES	Heart rate	$\bar{x}$	75	
		SD	8	
		SEM	3	

		↓ ½ hr Exercise				
		07h30	08h00	09h30	13h00	17h00
NON-ATHLETES						
Lactate	$\bar{x}$	1,23	1,64	0,76	0,73	0,61
	SD	0,16	1,20	0,35	0,62	0,31
	SEM	0,06	0,49	0,14	0,25	0,13
Ketones	$\bar{x}$	,055	,077	,064	,119	,079
	SD	,034	,048	,035	,063	,060
	SEM	,014	,019	,014	,026	,025
3HB/AA	$\bar{x}$	3,76	4,46	3,07	2,95	1,16
	SD	5,05	6,68	1,47	1,48	1,02
	SEM	2,06	2,73	0,60	0,61	0,42
ATHLETES						
Lactate	$\bar{x}$	0,81	0,89	0,77	0,64	0,91
	SD	0,21	0,46	0,17	0,21	0,55
	SEM	0.12	0,27	0,10	0,12	0,32
Ketones	$\bar{x}$	,051	,055	,044	,084	,083
	SD	,032	,032	,030	,045	,034
	SEM	,018	,018	,017	,026	,020
3HB/AA	$\bar{x}$ -	2,48	2,31	9,15	2,75	3,59
	SD	0,36	0,79	12,00	0,61	4,19
	SEM	0,20	0,45	6,93	0,35	2,42

		After ½hr of exercise
NON-ATHLETES		
Heart rate	$\bar{x}$	125
	SD	13
	SEM	5
ATHLETES		
Heart rate	$\bar{x}$	111
	SD	10
	SEM	6

↓ 1 hr Exercise

		07h00	08h00	09h30	13h00	17h00
NON-ATHLETES						
Lactate	$\bar{x}$	1,09	1,23	0,54	0,52	0,84
	SD	0,79	1,42	0,34	0,35	0,52
	SEM	0,35	0,63	0,15	0,16	0,23
Ketones	$\bar{x}$	,049	,077	,100	,151	,141
	SD	,025	,048	,057	,177	,129
	SEM	,011	,022	,026	,079	,058
3HB/AA	$\bar{x}$	7,38	8,18	5,35	3,00	4,30
	SD	9,31	12,2	3,95	1,28	2,97
	SEM	4,17	5,45	1,76	0,57	1,33
ATHLETES						
Lactate	$\bar{x}$	0,32	0,48	0,87	0,29	0,60
	½ range	0,17	0,14	0,39	0,11	0,27
Ketones	$\bar{x}$	,038	,045	,038	,053	,049
	½ range	,009	,010	,006	,012	,016
3HB/AA	$\bar{x}$	24,0	7,86	23,0	21,5	9,34
	½ range	22,0	4,14	20,0	19,5	6,15

		After ½ hr	After 1 hr of exercise
NON-ATHLETES			
Heart rate	$\bar{x}$	120	123
	SD	10	15
	SEM	4	7
ATHLETES			
Heart rate	$\bar{x}$	113	115
	½ range	7	13

↓ 1½ hrs Exercise

		06h30	08h00	09h30	13h00	17h00
NON-ATHLETES	$\bar{x}$	1,81	0,90	0,59	0,42	0,72
Lactate	SD	1,76	0,44	0,32	0,27	0,64
	SEM	0,72	0,18	0,13	0,11	0,26
	$\bar{x}$	,065	,140	,112	,100	,151
Ketones	SD	,042	,143	,103	,094	,165
	SEM	,017	,058	,042	,038	,067
	$\bar{x}$	8,67	8,43	3,08	1,59	2,87
3HB/AA	SD	10,4	8,01	1,51	1,12	3,31
	SEM	4,24	3,27	0,62	0,46	1,35
ATHLETES	$\bar{x}$	0,83	0,68	0,41	0,73	0,52
Lactate	SD	0,83	0,36	0,35	0,24	0,57
	SEM	0,48	0,21	0,20	0,14	0,33
	$\bar{x}$	,052	,087	,179	,153	,156
Ketones	SD	,034	,055	,198	,164	,189
	SEM	,019	,032	,114	,094	,109
	$\bar{x}$	13,2	6,23	2,15	2,87	1,93
3HB/AA	SD	20,6	3,02	0,52	0,85	1,01
	SEM	11,9	1,74	0,30	0,49	0,58

		After ½ hr	After 1½ hrs of exercise
NON-ATHLETES	$\bar{x}$	128	127
Heart rate	SD	10	14
	SEM	4	6
ATHLETES	$\bar{x}$	111	112
Heart rate	SD	9	11
	SEM	5	6

OLDER SUBJECTS

CONTROL

	Age yrs	Height cm	Mass kg		07h30	09h00	10h00	11h00	12h00	13h00	17h00
VS	51	170	65	Ketones							
				3HB/AA							
HW	41	187	66	Ketones	,006	,009	,032	,075		,043	,008
				3HB/AA	0,50	3,50	9,67	4,36		3,78	7,00
RL	38	183	61	Ketones	,052	,034	,117	,195	,270	,159	,010
				3HB/AA	2,71	1,27	3,50	3,64	3,58	2,38	2,33
EM	38	186	88	Ketones	,097	,119	,261	,220		,334	,192
				3HB/AA	1,78	2,22	3,83	5,28		4,39	3,68
JK	37	178	65	Ketones	,086	,142	,317	,205	,325	,361	,339
				3HB/AA	1,26	2,23	3,17	2,53	2,87	2,61	2,64
LK	30	186	90	Ketones							
				3HB/AA							

		↓1½ hrs 75W							HEART RATE		
		07h30	09h00	10h00	11h00	12h00	13h00	17h00	Before	½hr	After
VS	Ketones	,060	,124	,435	,484	,568	,517	,682	82	128	140
	3HB/AA	0,76	0,11	2,92	3,06	2,12	2,62	2,59			
HW	Ketones	,015	,022	,081	,188		,108	,021	90	140	150
	3HB/AA	0,50	3,40	9,12	4,37		3,69	6,00			
RL	Ketones	,110	,182	,380	,745		,736	,076	83	116	128
	3HB/AA	0,50	1,72	2,76	2,45		2,74	0,58			
EM	Ketones	,029	,118	,100	,113	,425	,276	,094	100	128	128
	3HB/AA	1,07	5,56	1,63	1,69	0,62	2,49	0,88			
JK	Ketones	,097	,555	,995	1,51	1,72	1,28	,754	80	136	136
	3HB/AA	1,78	2,88	3,67	4,90	5,73	4,11	3,43			
LK	Ketones	,038	,076	,155	,134	,219	,145	,142	92	112	112
	3HB/AA	0,90	4,43	1,54	2,72	2,84	1,54	1,18			

EFFECT OF DIET

NON-ATHLETE

Date: 12-12-78 Name: JK Age: 38 yrs Height: 178 cm Mass: 65 kg

CONTROL

Time	07h30	08h00	09h00	10h00	11h00	12h00	13h00	14h00	16h30
Ketones	,086	,112	,142	,317	,205	,325	,361	,237	,333
3HB/AA	1,26	1,67	2,23	3,17	2,53	2,87	2,61	3,39	2,54
FFA	0,49		0,56	0,85	0,81	0,71	0,75	0,74	1,01
Glucose	4,2	4,1	5,2	4,4	5,0	4,8	4,5	5,2	5,4
IRI	22	27	21	17	15	11	7,5	11	5,5
HGH	,6		<,5	,5	<,5	,7	,6	<,5	,5

JK Exercise 07h00 to 09h00 at 100 W NORMAL DIET

Date 23-1-79

	07h00	08h00	09h00	10h00	11h00	12h00	13h00	14h00	16h30
Ketones	,122	,543	,695	,606	1,17	1,10	1,26	,966	,630
3HB/AA	0,16	2,28	2,39	1,66	2,46	3,22	2,60	2,30	2,62
FFA	0,62	1,01	1,27	1,07	1,01	1,04	1,20	1,07	1,23
Glucose	4,1	4,6	4,7	4,5	5,4	4,8	4,6	5,7	5,4
IRI	25	4	1	<1	3,5	7,5	6,5	13,5	8
HGH	,5	,8	2,8	<,5	<,5	2,0	,6	,7	3,7

Date 26-2-79

Ketones	,130	,150	,398	,300	,348	,410	,325	,267	,436
3HB/AA	0,85	2,00	1,99	2,00	1,95	1,97	2,92	3,11	2,21
FFA	0,54	0,96	1,54	1,31	1,23	0,77	1,08	0,85	1,31
Glucose	4,1	5,3	5,0	4,9	5,7	5,2	5,2	5,1	5,2
IRI	13	12,5	8	4	10	10	7	8	2,5
HGH	<,5	3,1	,6	1,1	<,5	,6	,9	,7	,7

JK Exercise 07h00 to 09h00 at 100 W HIGH CARBOHYDRATE DIET FOR 2 DAYS

Date 30-1-79

	07h00	08h00	09h00	10h00	11h00	12h00	13h00	14h00	16h30
Ketones	,063	,117	,124	,132	,118	,173	,127	,060	,208
3HB/AA	2,15	1,78	0,88	1,00	1,23	1,84	2,26	1,14	1,97
FFA	0,48	0,69	0,92	0,95	0,92	0,86	0,92	0,92	1,14
Glucose	5,0	5,0	5,0	4,8	4,9	4,9	5,0	5,2	5,0
IRI	44	7	7	6	8	9	8,5	12	5
HGH	,7	,7	,7	<,5	<,5	,7	,6	<,5	,7

Date 5-2-79

Ketones	,091	,146	,271	,420	,383	,257	,473	,358	,257
3HB/AA	0,89	1,80	2,26	2,36	2,16	2,02	2,91	2,54	5,12
FFA	0,64	0,81	1,42	1,19	1,11	0,78	1,29	1,05	1,32
Glucose	4,4	4,9	4,8	5,0	5,5	5,1	4,9	5,3	5,2
IRI	57	12	12	8	12	11	11	13	12
HGH	,6	,7	,6	,8	,6	,7	,8	,7	,7

JK Exercise 07h00 to 09h00 at 100 W LOW CARBOHYDRATE DIET FOR 2 DAYS

Date 12-2-79

	07h00	08h00	09h00	10h00	11h00	12h00	13h00	14h00	16h30
Ketones	,129	,307	,715	1,59	1,69	1,37	1,20	1,72	2,18
3HB/AA	1,58	1,89	2,25	3,37	2,97	2,88	3,18	2,89	4,23
FFA	0,71	1,04	1,96	1,50	1,43	1,18	1,14	1,21	1,61
Glucose	4,3	4,8	4,5	4,5	4,3	4,4	4,3	5,0	4,8
IRI	21,5	7,5	2	1	<1	6,5	5	11	6,5
HGH	,5	,6	2,6	,5	,5	6,7	4,3	<,5	<,5

Date 19-2-79

Ketones	,239	,504	1,19	1,16	1,12	1,04	,711	,803	1,64
3HB/AA Ratio	2,46	2,47	3,22	2,68	2,73	3,45	1,05	2,11	3,13
FFA	0,80	1,72	2,40	1,24	1,20	1,40	1,56	1,20	1,64
Glucose	4,8	5,7	5,3	4,3	4,4	4,6	4,5	4,7	4,8
IRI	16	16	15	8	8	6	<1	4	4
HGH	<,5	1,0	1,3	<,5	,6	,8	1,2	1,0	,6

CONTROL

ATHLETES

		07h00	08h00	09h00	10h00	11h00	12h00	13h00	14h00	16h30
	Ketones	,110	,097	,067	,061	,069	,099	,046	,111	,294
TN	3HB/AA	0,75	0,94	0,81	0,85	0,86	1,54	1,09	1,09	1,75
29 yrs	FFA	0,60	0,69	0,69	0,75	0,80	1,01	1,06	1,10	1,25
187 cm	Glucose	4,5	5,0	4,5	4,6	4,9	5,0	4,9	4,9	5,0
77 kg	IRI	53	43	21	15,5	15	12	9	11	12,5
	HGH	,8	,6	,7	<,5	<,5	1,0	,8	4,4	<,5
	Ketones	,120	,123	,134	,216	,192	,180	,128	,153	,389
DC	3HB/AA	1,55	2,15	1,57	2,27	3,36	1,73	2,28	1,39	1,99
24 yrs	FFA	1,04	1,04	0,96	1,12	1,20	0,92	1,00	0,88	1,28
176 cm	Glucose	4,0	4,5	4,8	4,4	4,4	4,9	4,8	5,3	5,0
67 kg	IRI	7,5	7,5	5	6	6,5	4	3	12	13
	HGH	1,0	7,9	3,4	,9	,7	1,2	2,3	3,0	,9

RUN 07h00 - 09h00 at 12 km/h

NORMAL DIET

		07h00	08h00	09h00	10h00	11h00	12h00	13h00	14h00	16h00
TN	Ketones	,048	,072	,145	,248	,270	,210	,373	,558	,333
	3HB/AA	2,43	0,20	2,30	2,22	1,16	1,04	2,55	2,96	3,56
	FFA	0,73	0,93	1,22	1,05	1,05	1,09	1,13	1,09	1,09
	Glucose	4,4	6,1	5,9	5,5	5,2	5,5	4,8	5,2	4,9
	IRI	22	13	5,5	4,5	9	10	12,5	12,5	11
	HGH	,7	1,1	3,2	,9	,7	3,3	1,0	,6	,7
DC	Ketones	,056	,087	,235	,208	,244	,202	,081	,151	,187
	3HB/AA	0,87	1,17	1,53	1,67	2,48	1,30	2,52	1,32	1,20
	FFA	0,81	1,12	1,69	1,35	1,04	1,00	1,19	1,23	1,23
	Glucose	4,6	5,7	6,4	5,1	5,5	5,2	4,3	4,7	4,8
	IRI	8	3	4,5	9,5	3	<1	1	<1	7
	HGH	5,0	4,5	3,4	,8	,7	1,5	,7	,9	1,7

RUN 07h00 - 09h00 at 12 km/h

CARBOHYDRATE-FREE DIET

		07h00	08h00	09h00	10h00	11h00	12h00	13h00	14h00	16h30
TN	Ketones	,258	,331	,837	2,09	2,66	3,22	2,93	3,01	3,88
	3HB/AA	3,16	2,04	2,10	4,27	4,43	4,06	3,89	4,38	5,06
	FFA	0,51	1,09	1,66	2,17	1,63	1,46	1,36	1,36	1,32
	Glucose	4,9	5,9	4,0	4,9	4,2	4,7	4,2	4,2	4,0
	IRI	13,5	11	1	6	5	10	10	2	3
	HGH	,6	8,6	8,2	3,8	2,9	2,4	2,3	2,0	1,5
DC	Ketones	,365	,654	1,02	1,76	1,86	1,45	2,06	1,33	2,02
	3HB/AA	1,85	2,07	1,04	3,01	3,51	3,40	3,84	3,17	4,36
	FFA	1,05	1,73	2,05	2,08	1,39	1,61	1,36	1,33	1,77
	Glucose	4,0	5,3	4,8	4,5	4,8	4,3	4,2	4,5	4,1
	IRI	9	7	5	7,5	6	5	5	5	6,5
	HGH	2,5	1,8	4,0	,9	,9	1,2	1,4	3,6	1,4

EFFECT OF GLUCOSE
ALANINE & STARCH
ON
POST EXERCISE KETOSIS

SUBJECTS

	Age (yr)	Height (cm)	Mass (kg)
SD	20	191	64
JD	23	185	70
DC	24	176	67
TN	29	187	77
AFr	48	190	78
CS	22	180	65
ME	18	193	85
TS	20	185	75
GK	20	171	62
SM	24	180	57
JKr	22	175	74
RG	20	181	74
PJ	20	180	68
PS	22	173	63
BMa	33	176	70
JE	21	176	73
RC	37	176	66
DR	25	188	70
MS	35	178	77
JC	25	179	64

TOTAL KETONES

	07h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00	
SD	,182	,886	1,28	2,53	1,51	1,38	1,08	,100	1,18	CONTROL
JD	,193	,678	1,37	1,59	2,15	2,26	2,23	2,20	2,24	
DC	,365	1,02	1,76	1,86	1,45	2,06	1,33		2,02	
TN	,258	,837	2,08	2,66	3,22	2,93	3,01		3,88	
AFr	,746	1,28	2,05	2,39	1,93	1,56	2,12	1,67	2,19	
CS	,304	,366	1,40	1,75	1,53	1,88	1,59	1,64	1,44	
ME	1,10	2,11	2,77	3,73	4,03	1,68	1,06	1,43	2,30	100 g GLUCOSE at 11h10
TS	,293	,470	1,34	1,71	1,24	,216	,613	,970	1,14	
GK	,534	1,52	1,53	2,18	1,51	,249	,070	,499	,840	
DC	,059	,469	,713	,743	,172	,053	,084	,717		
SM	,285	,681	1,17	1,29	,972	,187	,065	,237	,460	
TN	,130	,432	1,04	1,20	,762	,090	,152	,796	,882	
JKr	,045	,172	,497	,483	,192	,364	,178	,271	,728	100 g ALANINE at 11h10
DC	,207	,355	,693	,814	,437	,065	,049	,330	,037	
RG	,770	1,56	2,31	2,43	1,80	,716	,718	,511	,444	
PJ	1,11	2,29	2,43	2,88	1,85	,365	,430	,309	,593	
PS	,536	,614	1,35	1,33	,123	,075	,053	,068	,060	
BMa	,307	1,28	1,00	1,78	,055	,020	,112	,036	,020	
JE	,846	,714	,775	,857	,403	,732	1,06	,428	,918	100 g STARCH at 11h10
CS	,428	,608	1,40	1,48	1,41	1,71	1,59	1,35	1,77	
RC	,108	,436	,598	1,17	1,30	1,01	1,00	1,00	,776	
DR	,080	,604	,986	1,29	,884	,690	,692	,540	,638	
MS	,090	,278	,636	,694	,640	,518	,484	,356	,494	
JC	,338	1,02	1,59	1,70	1,11	,540	,242	,443	,817	

3 HYDROXYBUTYRATE/ACETOACETATE RATIO

	07h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00	
SD	3,64	16,7	3,85	6,38	12,3	(192)	6,19	12,3	15,5	CONTROL
JD	0,74	6,60	11,8	16,1	14,4	19,5	28,8	25,8	17,4	
DC	1,85	1,04	3,31	3,50	3,40	3,84	3,17		4,36	
TN	3,16	2,10	4,28	4,43	4,06	3,98	4,38		5,06	
AFr	4,69	9,37	10,8	6,85	7,77	8,37	5,67	9,72	9,23	
CS	4,33	13,1	5,24	4,92	3,17	6,05	6,38	4,80	4,47	
ME	4,48	6,69	7,36	7,16	4,54	3,40	6,77	8,99	9,66	100 g GLUCOSE at 11h10
TS	11,2	20,4	5,44	5,04	3,36	5,97	2,35	2,48	3,59	
GK	4,34	2,16	5,08	5,25	1,94	4,53	16,5	6,68	5,46	
DC	3,21	9,42	5,04	3,29	4,21	1,21	41,0	5,09		
SM	1,30	2,18	2,33	2,53	1,93	1,49	1,03	1,28	2,13	
TN	1,71	3,91	5,53	4,69	3,82	1,65	2,30	4,68	15,3	
JKr	4,63	5,37	7,72	7,47	3,58	4,00	0,14	2,00	1,50	100 g ALANINE at 11h10
DC	1,46	2,09	2,59	2,60	2,62	8,38	2,30	1,83	1,92	
RG	2,38	3,78	4,80	5,10	3,47	2,58	3,30	3,68	2,08	
PJ	6,64	5,03	3,77	5,60	3,87	5,02	3,52	4,74	7,71	
PS	3,25	3,73	3,66	4,38	4,20	3,06	2,06	19,6	17,5	
BMa	0,76	7,11	4,51	48,5	0,32	0,39	0,19	0,55	2,85	
JE	3,84	3,35	2,58	2,81	1,76	2,25	3,13	1,93	8,88	100 g STARCH at 11h10
CS	7,92	6,07	8,33	5,51	8,25	8,42	9,43	8,23	7,37	
RC	1,84	2,11	2,44	3,05	3,66	2,35	1,67	2,94	1,92	
DR	3,44	1,58	3,14	2,74	2,23	1,67	3,49	2,18	2,54	
MS	3,09	2,39	3,68	2,24	1,69	2,32	2,84	2,71	2,53	
JC	5,04	2,10	3,27	4,04	2,85	2,35	2,61	1,93	3,00	

FREE FATTY ACIDS

	07h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00	
SD	0,77	2,50	2,55	1,95	1,32	1,55	1,73	1,64	2,50	CONTROL
JD		3,41	1,86	2,23	1,95	1,59	1,73	1,95	1,32	
DC	1,05	2,05	2,08	1,39	1,61	1,36	1,33		1,77	
TN	0,51	1,66	2,17	1,63	1,46	1,36	1,36		1,32	
AFr	1,13	2,33	1,72	1,62	1,56	2,03	1,72	1,62	2,13	
CS	1,25	2,19	1,81	1,56	1,31	1,69	1,50	1,25	1,56	
ME	1,97	2,64	2,33	2,90	1,62	1,41	2,13	2,13	2,28	100 g GLUCOSE at 11h10
TS	1,19	2,31	2,44	2,25	1,50	1,63	2,00	2,25	2,31	
GK	1,28	1,38	1,75	1,56	1,03	1,09	0,97	1,81	1,88	
DC	0,45	1,97	1,23	1,16	0,52	0,52	0,94	1,45		
SM	1,25	1,87	1,50	1,69	1,19	1,00	0,88	1,56	1,63	
TN	0,59	1,94	1,76	1,07	0,67	0,89	1,11	1,50	1,59	
JKr	0,74	2,10	1,32	1,32	0,74	0,87	1,00	1,26	1,06	100 g ALANINE at 11h10
DC	0,53	1,67	1,40	1,07	0,93	0,80	0,93	1,27	1,20	
RG	1,82	2,13	2,13	2,08	1,63	1,56	1,50	1,94	2,25	
PJ	1,19	2,66	1,47	1,44	1,87	1,62	1,87	1,90	1,51	
PS	1,14	1,77	2,04	1,55	1,31	1,09	1,16	1,22	1,53	
BMa	1,31	2,31	1,69	2,13	0,95	1,14	0,68	1,00	1,73	
JE	1,55	1,59	1,50	1,77	1,73	1,41	1,68	1,45	1,68	100 g STARCH at 11h10
CS	1,72	1,80	2,46	1,33	1,67	1,24	1,41	1,33	1,85	
RC	2,23	2,73	2,09	2,27	1,55	1,36	1,73	2,73	2,00	
DR	0,91	2,27	2,36	2,00	1,55	1,50	1,91	1,55	1,27	
MS	1,64	2,55	1,45	1,45	2,00	2,09	1,91	1,82	2,14	
JC	1,20	1,80	1,70	1,65	1,50	1,35	1,30	1,35	1,75	

GLUCOSE

	07h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00	
SD	5,2	6,1	5,7	5,9	5,7	5,5	5,5	5,2	5,3	CONTROL
JD	4,0	4,8	4,8	4,8	5,2	5,0	5,3	4,8	4,9	
DC	4,0	4,8	4,5	4,8	4,3	4,2	4,5		4,1	
TN	4,9	4,0	4,9	4,2	4,7	4,2	4,2		4,0	
AFr	4,4	4,7	4,8	5,6	4,6	5,0	5,7	4,8	5,3	
CS	5,4	4,0	4,4	4,2	4,4	4,2	4,9	4,5	5,0	
ME	5,2	4,7	4,4	5,2	13,5	6,8	5,3	4,2	4,4	100 g GLUCOSE at 11h10
TS	4,9	2,9	4,2	4,4	8,7	4,9	3,9	4,2	4,4	
GK	4,5	3,6	4,4	4,4	9,6	8,8	7,2	4,5	4,6	
DC	3,7	4,4	4,4	4,5	9,6	6,1	2,0	3,7		
SM	4,5	3,9	5,0	4,8	11,3	10,6	8,1	4,3	4,9	
TN	5,5	3,9	4,5	5,0	11,4	7,2	4,1	4,1	4,3	
JKr	4,5	5,3	4,9	4,9	5,6	5,4	5,8	5,9	6,2	100 g ALANINE at 11h10
DC	5,2	5,7	5,0	5,7	5,5	5,2	5,2	6,2	6,2	
RG	4,8	4,4	4,9	4,7	5,4	5,3	6,6	7,6	6,9	
PJ	5,1	4,1	4,4	4,9	6,2	6,7	8,2	8,6	7,2	
PS	4,8	4,4	4,4	4,3	4,9	4,9	5,3	5,8	5,8	
BMa	5,5	5,0	4,9	5,5	5,8	5,4	5,8	5,8	6,2	
JE	4,8	4,8	4,6	5,0	5,7	4,8	4,8	4,6	4,5	100 g STARCH at 11h10
CS	4,1	3,1	3,6	5,0	4,8	4,4	3,9	4,0	4,0	
RC	6,5	4,5	4,5	5,0	5,2	5,0	4,8	4,7	4,9	
DR	6,5	4,4	5,0	4,8	5,2	5,0	4,9	4,8	4,9	
MS	4,9	4,5	4,6	4,4	4,8	4,7	4,9	4,7	4,9	
JC	5,2	3,9	5,5	5,7	8,5	8,0	6,6	5,5	5,2	

INSULIN

	07h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00	
SD	20	25	22	23	17	16,5	17	18,5	17	CONTROL
JD	28	19	20	23	26	27	32	29	30	
DC	9	5	7,5	6	5	5	5		6,5	
TN	13,5	1	6	5	10	10	2		3	
AFr										
CS	6	1	2,5	3	0	0	0	0	1	
ME	8,5	7	8	8,5	87	66	18	10	7,5	100 g GLUCOSE at 11h10
TS	6	0	0	0	29	4,5	0	0	0	
GK	8	3	4	5	18	22	20,5	8	7	
DC	8	4	4,5	5	26	32	7	5		
SM	10	9	9	7	66	88	62	9	6	
TN	32	11	12	16	68	42	15	12	12,5	
JKr	11	12	8,5	13,5	20	16,4	11	16	14	100 g ALANINE at 11h10
DC	13	5	5	8	10	8	9	8	8	
RG	0	0	1,5	2	10	6	7	8	7	
PJ	8	4	5	7	11,5	17,5	23	23	14	
PS	6,5	3,5	4	4	5	5	5,5	6,5	5,5	
BMa	18	14	15	18	18,5	15	16	17	16	
JE	11	12	14	16,5	18	16	15	12	14	100 g STARCH at 11h10
CS	16	11	12	15	16	14,5	14	16	13	
RC	26	12	12	12	14	14	13	20	15	
DR	37	11	11	13	16,5	15	16	16	17	
MS	26	13	14,5	14	14	12	13,5	13	12,5	
JC	10	4,5	7	8	26	28,5	13,5	9	8	

GLUCAGON

	07h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00	
SD	23	44	52	45	21	<20	<20	<20	<20	CONTROL
JD	25	98	114	50	47	54	47	39	28	
DC	153	238	173	121	105	113	96		125	
TN	157	136	81	78	72	49	70		56	
AFr	162	173	159	88	94	74	81	<20	<20	
CS	110	186	177	150	115	157	125	174	144	
ME	278	781	369	<20	<20	54	43	63	72	100 g GLUCOSE at 11h10
TS	76	256	125	103	66	86	58	95	95	
GK	163	273	165	147	121	168	158	182	174	
DC	147	165	97	86	39	37	64	90		
SM	38	40	26	24		23	<20	32	27	
TN	44	250	65	22	<20	<20	<20	35	<20	
JKr	96	110	75	87	179	158	191	182	142	100 g ALANINE at 11h10
DC	103	142	92	82	250	253	264	180	252	
RG	101	95	77	44	131	212	219	124	42	
PJ	81	199	173	189	261	220	228	271	266	
PS	140	222	116	95	204	485	325	227	108	
BMa	39	53	47	40	132	180	193	233	80	
JE	44	419	354	170	98	121	115	121	70	100 g STARCH at 11h10
CS	79	277	161	124	78	85	77	81	68	
RC	35	87	63	55	22	24	46	31	60	
DR	162	608	305	138	78	90	62	78	78	
MS	41	82	86	81	44	37	37	39	70	
JC	<20	332	76	46	<20	<20	<20	<20	<20	

GROWTH HORMONE

	07h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00	
SD	0,77	8,74	3,31	0,87	0,84	1,20	1,56	1,80	3,47	CONTROL
JD	0,55	13,26	4,82	2,60	9,62	4,93	5,00	3,98	2,24	
DC	2,53	3,97	0,86	0,86	1,24	1,37	3,61		1,42	
TN	0,60	8,20	3,80	2,90	2,40	2,30	2,00		1,50	
AFr	0,56	7,87	1,66	0,99	2,25	1,22	1,05	0,92	1,44	
CS	0,67	<0,5	11,5	9,69	5,95	7,40	3,07	2,48	6,20	
ME	0,70	5,11	3,79	1,64	0,77	0,72	<0,5	0,73	1,29	100 g GLUCOSE at 11h10
TS	0,67	<0,5	7,86	4,86	4,94	1,13	1,97	2,60	2,38	
GK	1,03	10,28	4,19	4,15	2,48	1,32	3,29	<0,5	1,18	
DC	3,21	3,06	1,19	1,89	2,60	3,89	6,94	4,18		
SM	0,66	9,97	1,97	0,77	0,62	0,65	<0,5	8,04	7,67	
TN	<0,5	14,83	8,82	6,94	7,53	1,39	6,43	6,70	6,93	
JKr	<0,5	14,4	1,51	<0,5	1,77	13,98	10,65	4,26	6,05	100 g ALANINE at 11h10
DC	2,90	2,78	1,02	1,64	3,07	6,50	4,58	1,60	1,76	
RG	0,96	10,0	10,6	4,28	5,35	9,53	12,9	7,59	7,97	
PJ	<0,5	5,69	3,39	3,59	7,08	15,36	5,25	7,64	1,78	
PS	0,97	7,76	6,41	4,03	2,61	13,9	9,46	5,64	4,19	
BMa	0,76	1,32	0,88	0,76	1,00	7,06	17,4	8,34	1,80	
JE	3,99	8,02	7,66	1,89	0,90	1,04	3,47	2,91	1,90	100 g STARCH at 11h10
CS	5,48	>20	>20	>20	>20	16,23	12,94	11,47	13,96	
RC	0,72	12,1	6,38	3,41	12,1	13,1	9,62	3,46	1,24	
DR	<0,5	14,51	14,56	8,03	10,42	1,84	0,52	5,21	0,59	
MS	0,5	13,31	2,81	0,51	0,49	0,61	<0,5	3,23	0,56	
JC	0,58	14,4	6,88	3,77	3,22	0,89	4,61	7,62	2,81	

KETONES		07h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00
	$\bar{x}$	,412	,896	1,30	1,81	1,94	1,96	2,00	1,81	2,16
Control	SD	,324	,630	,691	,808	,429	,698	,625	,710	,947
	SEM	,067	,148	,158	,190	,175	,285	,255	,290	,386
	$\bar{x}$				1,81	1,45	,412	,341	,775	1,13
Glucose*	SD				1,06	1,34	,624	,412	,409	,701
	SEM				,432	,549	,255	,168	,167	,314
	$\bar{x}$				1,62	,742	,268	,257	,254	,314
Alanine*	SD				,926	,848	,268	,266	,177	,314
	SEM				,378	,346	,109	,108	,072	,128
	$\bar{x}$	,315	,610	,998	1,20	,956	,865	,844	,686	,903
Starch*	SD	,298	,253	,414	,378	,388	,447	,477	,398	,452
	SEM	,122	,103	,169	,154	,158	,182	,195	,162	,184

3HB/AA										
	$\bar{x}$	3,54	6,73	5,15	7,97	7,59	8,28	9,14	10,0	9,34
Control	SD	2,50	5,30	2,81	10,5	4,78	6,57	9,67	8,48	5,83
	SEM	0,59	1,25	0,66	2,49	1,95	2,94	3,95	3,46	2,38
	$\bar{x}$				4,66	3,30	3,04	11,7	4,87	7,23
Glucose*	SD				1,62	1,13	1,93	15,5	2,79	5,32
	SEM				0,66	0,46	0,79	6,31	1,14	2,38
	$\bar{x}$				5,00	3,01	3,91	1,92	5,41	5,59
Alanine*	SD				1,59	1,42	2,69	1,47	7,12	6,27
	SEM				0,65	0,58	1,10	0,60	2,91	2,56
	$\bar{x}$	4,20	2,93	3,90	3,40	3,41	3,23	3,86	3,32	4,37
Starch*	SD	2,10	1,64	2,22	1,19	2,49	2,56	2,80	2,44	2,96
	SEM	0,86	0,67	0,91	0,49	1,01	1,04	1,14	1,00	1,21

* Refers to substance taken at 11h10

FFA		07h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00
	$\bar{x}$	1,07	2,11	1,82	1,77	1,53	1,65	1,56	1,52	1,77
Control	SD	0,43	0,53	0,38	0,49	0,24	0,20	0,18	0,26	0,47
	SEM	0,11	0,12	0,09	0,12	0,10	0,08	0,07	0,11	0,19
	$\bar{x}$				1,30	1,09	1,09	1,34	1,78	1,94
Glucose*	SD				0,33	0,44	0,39	0,57	0,34	0,35
	SEM				0,19	0,18	0,16	0,23	0,14	0,16
	$\bar{x}$				1,60	1,24	1,18	1,19	1,43	1,55
Alanine*	SD				0,42	0,44	0,34	0,43	0,39	0,42
	SEM				0,17	0,18	0,14	0,18	0,16	0,17
	$\bar{x}$	1,54	2,12	1,93	1,75	1,67	1,49	1,66	1,70	1,78
Starch*	SD	0,45	0,46	0,44	0,35	0,18	0,31	0,25	0,53	0,30
	SEM	0,18	0,19	0,18	0,14	0,08	0,12	0,10	0,22	0,12

GLUCOSE										
	$\bar{x}$	4,8	4,6	4,6	4,9	4,8	4,8	5,0	4,7	4,8
Control	SD	0,5	0,9	0,4	0,5	0,6	0,5	0,7	0,3	0,6
	SEM	0,1	0,2	0,1	0,1	0,2	0,2	0,3	0,1	0,1
	$\bar{x}$				4,7	10,7	7,4	5,1	4,2	4,5
Glucose*	SD				0,3	1,7	2,1	2,3	0,3	0,3
	SEM				0,1	0,7	0,8	0,9	0,1	0,1
	$\bar{x}$				5,0	5,6	5,5	6,2	6,6	6,4
Alanine*	SD				0,5	0,4	0,6	1,1	1,2	0,5
	SEM				0,2	0,2	0,3	0,5	0,5	0,2
	$\bar{x}$	5,3	4,2	4,6	5,0	5,7	5,3	5,0	4,7	4,7
Starch*	SD	1,0	0,6	0,6	0,4	1,4	1,3	0,9	0,5	0,4
	SEM	0,4	0,3	0,3	0,2	0,6	0,6	0,4	0,2	0,2

* Refers to substance taken at 11h10

IRI		07h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00
	$\bar{x}$	12	8	7	9	11	12	13	11	11
Control	SD	8	7	6	7	11	11	12	12	12
	SEM	2	2	2	2	5	5	6	6	5
	$\bar{x}$				7	49	42	20	7	7
Glucose*	SD				5	28	30	22	4	4
	SEM				2	12	12	9	2	2
	$\bar{x}$				9	12	11	12	13	11
Alanine*	SD				6	6	6	7	7	4
	SEM				2	2	2	3	3	2
	$\bar{x}$	21	11	12	13	17	17	14	14	13
Starch*	SD	11	3	3	3	4	6	1	4	3
	SEM	4	1	1	1	2	2	1	2	1

IRG										
	$\bar{x}$	108	184	128	84	79	79	71	67	62
Control	SD	65	165	81	53	39	49	44	63	59
	SEM	15	39	19	12	16	20	18	26	24
	$\bar{x}$				65	49	63	57	83	76
Glucose*	SD				55	46	58	54	55	65
	SEM				22	21	24	22	23	29
	$\bar{x}$				70	179	258	238	189	125
Alanine*	SD				26	50	132	57	44	80
	SEM				11	23	59	25	20	36
	$\bar{x}$	61	301	174	102	55	61	58	60	59
Starch*	SD	54	202	126	49	35	44	36	41	25
	SEM	22	82	51	20	14	18	15	17	10

* Refers to substance taken at 11h10

HGH		07h00	09h00	10h00	11h00	12h00	13h00	14h00	15h00	16h00
	$\bar{x}$	1,01	6,99	4,73	2,98	3,74	3,07	2,39	2,47	2,71
Control	SD	0,90	4,74	3,40	2,48	3,44	2,57	1,47	1,15	1,88
	SEM	0,21	1,12	0,80	0,59	1,40	1,05	0,60	0,47	0,77
	$\bar{x}$				3,38	3,16	1,52	3,17	3,74	3,89
Glucose*	SD				2,35	2,65	1,20	2,96	3,17	3,16
	SEM				0,96	1,08	0,49	1,21	1,30	1,41
	$\bar{x}$				2,42	3,48	11,06	10,04	5,85	3,93
Alanine*	SD				1,77	2,30	3,85	4,81	2,57	2,64
	SEM				0,72	0,94	1,57	1,96	1,05	1,08
	$\bar{x}$	1,91	15,39	11,38	7,94	9,52	5,62	5,23	5,65	3,51
Starch*	SD	2,24	7,55	9,89	11,1	11,16	7,09	5,09	3,34	5,19
	SEM	0,92	3,08	4,04	4,53	4,55	2,89	2,08	1,37	2,12

* Refers to substance taken at 11h10

65 HOUR FAST

KETONES

	Age yrs	Height cm	Mass kg	11h00	12h00	13h00	14h00	15h00	16h00	
BH	18	172	62	,702	,454	,686	,734	,732	,680	CONTROL
PM	18	167	59	5,18	1,84	1,70	3,25	3,08	5,29	
MF	19	189	73	2,17	1,95	2,21	2,79	2,41	2,92	
MA	19	182	70	4,01	3,94	4,72	4,75	4,31	4,56	
TC	25	167	62	1,54	,740	,192	,052	,066	,350	100g GLUCOSE at 11h10
MW	19	175	63	,894	1,12	,162	,070	,108	,604	
KL	27	184	85	1,23	1,23	,792	,094	,084	,430	
NH	19	173	68	4,06	2,67	2,64	2,76	2,91	4,33	100g STARCH at 11h00
JM	19	166	66	,586	,526	,700	,848	1,06	1,09	
RJ	19	173	62	1,50	1,38	1,28	1,37	1,26	1,41	
MD	19	178	70	4,67	4,06	2,55	2,06	3,01	3,14	100g ALANINE at 11h10
GM	21	188	72	1,47	,642	,314	,172	,428	1,35	

3HB/AA

	11h00	12h00	13h00	14h00	15h00	16h00
BH	4,85	4,04	8,03	4,92	3,88	5,18
PM	9,37	5,75	6,46	4,51	7,45	5,39
MF	5,26	5,80	4,05	7,19	7,10	7,06
MA	4,01	5,00	3,79	4,15	4,16	3,80
TC	5,02	3,40	3,17	0,86	0,37	2,80
MW	6,58	2,13	1,61	0,84	1,07	5,04
KL	9,05	4,24	3,60	10,8	3,67	7,60
NH	7,64	6,46	5,92	6,59	8,84	6,76
JM	3,37	3,31	5,03	2,69	3,30	3,32
RJ	3,83	2,68	2,91	3,51	3,21	3,41
MD	4,81	2,74	2,56	2,43	3,08	3,60
GM	3,63	2,24	3,03	2,50	1,97	2,95

FFA

	11h00	12h00	13h00	14h00	15h00	16h00
BH	2,05	1,76	2,05	2,14	2,05	2,14
PM	2,17	1,74	1,65	1,48	1,83	2,09
MF	2,43	2,14	2,14	2,24	2,33	2,62
MA	2,87	1,74	1,74	2,26	2,17	2,26
TC	1,86	1,52	1,05	1,19	1,19	1,95
MW	2,33	1,29	0,95	1,10	1,33	2,43
KL	2,35	1,13	1,00	0,87	0,96	1,74
NH	2,00	1,22	1,91	1,74	1,91	2,26
JM	1,83	1,39	1,65	1,04	1,65	1,83
RJ	2,09	1,39	1,65	2,22	1,65	1,70
MD	2,09	2,35	1,39	2,43	3,30	2,17
GM	2,43	1,30	1,87	1,48	1,57	2,09

CONTROL

100g GLUCOSE at 11h10

100g STARCH at 11h10

100g ALANINE at 11h10

	GLUCOSE						IRI						
	11h00	12h00	13h00	14h00	15h00	16h00	11h00	12h00	13h00	14h00	15h00	16h00	
BH	4,9	4,9	5,1	5,4	5,5	5,5	8	8	8	8	9	11	CONTROL
PM	3,4	3,6	3,6	3,4	3,5	3,6	16	16	12,5	12	10,5	9	
MF	3,6	3,6	3,2	3,2	3,3	3,2	8	8	8	8	8	7	
MA	3,2	3,7	3,6	3,6	3,5	3,5	10	10	8	9	11	12	
TC	4,7	8,7	11,4	6,2	8,1	3,0	12	34	177	120	132	19	100g GLUCOSE at 11h10
MW	5,2	10,4	8,7	7,6	5,5	4,3	9	68	50	32	12	9	
KL	4,5	8,2	11,3	9,0	7,1	3,9	12	48	97	79	35,5	20	
NH	3,7	4,4	3,9	4,0	3,8	3,9	6	9	8	5	5	4	100g STARCH at 11h10
JM	5,5	5,9	5,4	5,3	5,3	5,1	13	12	9	10	9	11	
RJ	4,4	5,0	4,7	4,5	4,5	4,4	15	14,5	14,5	24	18,5	13	
MD	3,6	5,2	7,2	6,7	5,6	4,6	12,5	31	28	19	18	15	100g ALANINE at 11h10
GM	4,4	5,6	6,6	7,4	6,4	5,9	13	24	18	28	21	14	

IRG

HGH

	11h00	12h00	13h00	14h00	15h00	16h00	11h00	12h00	13h00	14h00	15h00	16h00	
BH	83	51	46	56	39	50	1,68	1,18	2,30	3,11	2,84	0,89	CONTROL
PM	101	84	108	68	99	94	5,17	12,29	6,18	8,12	8,11	6,15	
MF	152	162	154	180	178	164	3,76	2,94	3,25	2,46	4,59	4,27	
MA	494	473	437	388	470	379	4,34	5,50	3,04	2,39	2,91	1,60	
TC	93	48	45	42	23	37	6,61	1,27	0,62	0,64	0,61		GLUCOSE at 11h10
MW	49	42	37	43	32	62	1,01	0,67	0,63	0,61	2,58	0,81	
KL	53	35	<20	<20	23	40	0,86	<0,5	0,63	3,80	1,21	0,85	
NH	344	270	288	577	424	555	0,86	2,29	2,62	0,91	1,71	0,72	STARCH at 11h10
JM	656	661	206	189	195	215	<0,5	>20,0	4,76	0,80	0,68	2,21	
RJ	112	116	58	73	55	64	4,35	7,89	3,01	3,56	2,24	7,35	
MD	118	437	345	101	149	98	5,35	6,75	>20,0	14,29	2,17	0,94	ALANINE at 11h10
GM	130	395	535	435	128	112	8,33	5,49	9,02	>20,0	9,35	1,08	

KETONES		11h00	12h00	13h00	14h00	15h00	16h00
Control	$\bar{x}$	2,19	2,05	2,33	2,88	2,63	3,36
	SD	1,63	1,43	1,71	1,66	1,49	2,04
	SEM	0,52	0,72	0,86	0,83	0,74	1,02
Glucose*	$\bar{x}$	1,22	1,03	0,38	0,07	0,09	0,46
	SD	0,32	0,26	0,35	0,02	0,02	0,12
	SEM	0,19	0,15	0,20	0,01	0,01	0,07
Alanine*	$\bar{x}$	3,07	2,35	1,43	1,12	1,72	2,25
	½ range	1,60	1,71	1,12	0,94	1,29	0,89
Starch*	$\bar{x}$	2,05	1,52	1,54	1,66	1,74	2,28
	SD	1,80	1,08	1,00	1,00	1,01	1,79
	SEM	1,00	0,62	0,57	0,57	0,59	1,03

3HB/AA							
Control	$\bar{x}$	5,90	5,15	5,58	5,19	5,65	5,36
	SD	2,16	0,82	2,03	1,37	1,89	1,34
	SEM	0,68	0,41	1,01	0,68	0,94	0,67
Glucose*	$\bar{x}$	6,88	3,26	2,79	4,17	1,70	5,15
	SD	2,03	1,06	1,05	5,74	1,74	2,40
	SEM	1,17	0,61	0,60	3,32	1,00	1,39
Alanine*	$\bar{x}$	4,22	2,49	2,80	2,47	2,53	3,28
	½ range	0,59	0,25	0,24	0,04	0,56	0,33
Starch*	$\bar{x}$	4,95	4,15	4,62	4,26	5,12	4,50
	SD	2,34	2,03	1,55	2,06	3,22	1,96
	SEM	1,35	1,17	0,89	1,19	1,86	1,13

* Refers to substance taken at 11h10

FFA		11h00	12h00	13h00	14h00	15h00	16h00
Control	$\bar{x}$	2,20	1,85	1,90	2,03	2,10	2,28
	SD	0,31	0,20	0,24	0,37	0,21	0,24
	SEM	0,10	0,10	0,12	0,19	0,11	0,12
Glucose*	$\bar{x}$	2,18	1,31	1,00	1,05	1,16	2,04
	SD	0,28	0,20	0,05	0,17	0,19	0,35
	SEM	0,16	0,11	0,03	0,10	0,11	0,20
Alanine*	$\bar{x}$	2,26	1,83	1,63	1,96	2,44	2,13
	$\frac{1}{2}$ range	0,17	0,53	0,24	0,48	0,86	0,04
Starch*	$\bar{x}$	1,97	1,33	1,74	1,67	1,74	1,93
	SD	0,13	0,10	0,15	0,59	0,15	0,29
	SEM	0,08	0,06	0,09	0,34	0,09	0,17

GLUCOSE							
Control	$\bar{x}$	4,31	3,93	3,88	3,90	3,95	3,95
	SD	0,80	0,66	0,84	1,01	1,04	1,05
	SEM	0,25	0,33	0,42	0,51	0,52	0,52
Glucose*	$\bar{x}$	4,80	9,10	10,47	7,60	6,90	3,73
	SD	0,36	1,15	1,53	1,40	1,31	0,67
	SEM	0,21	0,67	0,88	0,81	0,76	0,38
Alanine*	$\bar{x}$	4,00	5,40	6,90	7,05	6,00	5,25
	$\frac{1}{2}$ range	0,40	0,20	0,30	0,35	0,40	0,65
Starch*	$\bar{x}$	4,53	5,10	4,67	4,60	4,53	4,47
	SD	0,91	0,75	0,75	0,66	0,75	0,60
	SEM	0,52	0,44	0,43	0,38	0,43	0,35

* Refers to substance taken at 11h10

IRI		11h00	12h00	13h00	14h00	15h00	16h00
Control	$\bar{x}$	11	10	9	9	10	10
	SD	3	4	2	2	1	2
	SEM	1	2	1	1	1	1
Glucose*	$\bar{x}$	11	50	108	77	60	16
	SD	2	17	64	44	64	6
	SEM	1	10	37	25	37	4
Alanine*	$\bar{x}$	13	27	23	23	19	14
	½ range	0	4	5	4	1	1
Starch*	$\bar{x}$	11	12	10	13	11	9
	SD	5	3	3	10	7	5
	SEM	3	2	2	6	4	3

IRG							
Control	$\bar{x}$	214	193	186	173	197	172
	SD	212	193	173	154	191	146
	SEM	67	96	86	77	96	73
Glucose*	$\bar{x}$	65	42	31	32	26	46
	SD	24	7	18	19	5	14
	SEM	14	4	11	11	3	8
Alanine*	$\bar{x}$	124	416	440	268	139	105
	½ range	6	21	95	167	11	7
Starch*	$\bar{x}$	371	349	184	280	225	278
	SD	273	281	117	264	186	252
	SEM	158	162	67	154	108	145

* Refers to substance taken at 11h10

HGH		11h00	12h00	13h00	14h00	15h00	16h00
Control	$\bar{x}$	3,54	5,48	3,69	4,02	4,61	3,23
	SD	2,61	4,88	1,71	2,75	2,47	2,43
	SEM	0,75	2,44	0,85	1,38	1,23	1,22
Glucose*	$\bar{x}$	2,83	0,71	0,63	1,68	1,47	0,83
	SD	3,28	0,54	0,01	1,83	1,01	0,03
	SEM	1,89	0,31	0	1,06	0,58	0,02
Alanine*	$\bar{x}$	6,84	6,12	19,51	22,15	5,76	1,01
	$\frac{1}{2}$ range	1,49	0,63	10,49	7,85	3,59	0,07
Starch*	$\bar{x}$	1,80	13,39	3,46	1,76	1,54	3,43
	SD	2,23	14,65	1,14	1,56	0,79	3,48
	SEM	1,29	8,46	0,66	0,90	0,46	2,01

* Refers to substance taken at 11h10

EFFECT OF ALANINE AND GLUCOSE ON NON-FASTING SUBJECTS

KETONES

	Age	Height	Mass	11h00	12h00	13h00	14h00	15h00	16h00	
DS	19	174	73	,398	,332	,350	,498	,576	,416	CONTROL
JC	18	178	83	,068	,059	,076	,089	,130	,094	
AP	18	190	87	,052	,076	,212	,198	,150	,194	
BMc	18	183	69	,140	,216	,478	,510	,318	,786	
JN	19	183	87	,062	,038	,050	,034	,028	,034	ALANINE AT 11h10
HB	20	193	88	,112	,050	,070	,047	,082	,062	
DF	19	187	75			,064	,046	,064	,048	
SS	20	173	69	,070	,056	,052	,052	,064	,060	GLUCOSE AT 11h10
AD	20	183	69	,046	,040	,088	,042	,076	,164	
BS	18	169	72	,050	,074	,046	,044	,058	,070	
BH	18	172	62	,044	,042	,046	,048	,056	,188	

	GLUCOSE						IRI						
	11h00	12h00	13h00	14h00	15h00	16h00	11h00	12h00	13h00	14h00	15h00	16h00	
DS	5,5	6,5	5,6	5,5	5,5	5,3	17	13	17	13,5	12,5	13	CONTROL
JC	6,0	5,9	6,0	5,8	5,7	6,0	25	10	21	22	48	25	
AP	4,7	5,4	5,5	5,4	5,5	5,5	15	10	14	13	10	13	
BMc	6,0	6,1	6,0	6,3	6,1	6,2	17	17	14	19	10	16	
JN	6,7	6,0	5,8	6,4	6,2	6,5	21,5	28	23	22	23	20	ALANINE AT 11h10
HB	6,2	5,1	5,4	5,9	6,2	6,2	19,5	20,5	17,5	17	22	17	
DF	5,9	5,7	6,1	6,7	6,4	6,3	21,5	45	28,5	22	21	20	
SS	6,8	6,0	6,7	6,1	6,0	6,0	84	105	53	50	27	22	GLUCOSE AT 11h10
AD	6,4	6,3	6,7	4,0	5,3	5,3	116	83	110	29	15,5	19	
BS	5,4	5,2	4,5	4,9	5,2	5,4	16	31	28	19	10	10	
BH	5,5	9,6	6,4	6,5	6,0	6,1	11	44	47	26	13	9	

IRG	11h00	12h00	13h00	14h00	15h00	16h00	3HB/AA 11h00	12h00	13h00	14h00	15h00	16h00	
DS	48	71	71	58	62	65	10,1	5,64	4,65	8,96	7,00	7,88	CONTROL
JC	103	91	85	68		58	1,61	1,11	0,81	2,18	1,83	1,61	CONTROL
AP	135	129	142	119	133	139	7,67	4,43	3,82	5,60	7,33	2,35	CONTROL
BMc	74	40	68	41	45	20	1,19	1,40	1,60	2,07	1,94	2,45	CONTROL
JN	134	114	135	136	102	99	1,21	0,73	0,56	0,55	0,56	0,89	ALANINE AT 11h10
HB	27	93	70	71	58	65	3,67	1,08	2,50	6,67	4,86	2,88	ALANINE AT 11h10
DF	41	146	131	146	137	124			1,91	4,75	3,00	23,0	ALANINE AT 11h10
SS	80	66	43	61	20	32	1,19	1,33	0,86	1,16	0,88	0,76	GLUCOSE AT 11h10
AD	74	60	21	46	42	32	0,15	0,05	0,42	0,22	1,00	1,41	GLUCOSE AT 11h10
BS	124	129	121		151	121	0,25	0,29	0,35	0,29	0,16	0,25	GLUCOSE AT 11h10
BH	<20	<20	<20	<20	<20	<20	0,05	0,05	0,15	0,33	0,17	1,41	GLUCOSE AT 11h10

KETONES		11h00	12h00	13h00	14h00	15h00	16h00
	$\bar{x}$	,104	,171	,279	,324	,293	,372
Control	SD	,108	,128	,173	,213	,206	,307
	SEM	,034	,064	,087	,106	,103	,153
	$\bar{x}$	,052	,053	,058	,046	,063	,120
Glucose*	SD	,012	,016	,020	,004	,009	,065
	SEM	,006	,008	,010	,002	,004	,032
	$\bar{x}$	,087	,044	,061	,042	,058	,048
Alanine*	SD	,035	,008	,010	,007	,027	,014
	SEM	,025	,006	,006	,004	,016	,008

GLUCOSE							
	$\bar{x}$	5,92	5,98	5,78	5,75	5,70	5,75
Control	SD	,62	,46	,26	,40	,28	,42
	SEM	,19	,23	,13	,20	,14	,21
	$\bar{x}$	6,03	6,78	6,08	5,38	5,63	5,70
Glucose*	SD	,68	1,94	1,06	1,14	,43	,41
	SEM	,34	,97	,53	,57	,22	,20
	$\bar{x}$	6,27	5,60	5,77	6,33	6,27	6,33
Alanine*	SD	,40	,46	,35	,40	,12	,15
	SEM	,23	,26	,20	,23	,07	,09

IRI							
	$\bar{x}$	33	12	16	17	20	17
Control	SD	34	3	3	4	19	6
	SEM	10	2	2	2	9	3
	$\bar{x}$	57	66	59	31	16	15
Glucose*	SD	52	34	35	13	7	6
	SEM	26	17	18	7	4	3
	$\bar{x}$	21	31	23	20	22	19
Alanine*	SD	1	13	5	3	1	2
	SEM	1	7	3	2	1	1

IRG		11h00	12h00	13h00	14h00	15h00	16h00
	$\bar{x}$	77	83	92	72	80	71
Control	SD	43	37	34	34	47	50
	SEM	13	19	17	17	27	25
	$\bar{x}$	72	66	49	39	56	49
Glucose*	SD	47	49	50	26	65	49
	SEM	23	24	25	15	32	25
	$\bar{x}$	67	118	112	118	99	96
Alanine*	SD	58	27	36	41	40	30
	SEM	34	15	21	24	23	17

3HB/AA							
	$\bar{x}$	2,71	3,15	2,72	4,70	4,53	3,57
Control	SD	3,46	2,24	1,81	3,28	3,05	2,90
	SEM	1,09	1,12	0,91	1,64	1,53	1,45
	$\bar{x}$	0,41	0,43	0,45	0,50	0,55	0,96
Glucose*	SD	0,53	0,61	0,30	0,44	0,45	0,56
	SEM	0,26	0,31	0,15	0,22	0,23	0,28
	$\bar{x}$	2,44	0,91	1,66	3,99	2,81	8,92
Alanine*	SD	1,74	0,25	0,99	3,13	2,16	12,23
	SEM	1,23	0,18	0,57	1,81	1,25	7,06

* Refers to substance taken at 11h10

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